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A CYTOLOGICAL STUDY OF PUCCINIA TRITICINA PHYSIOLOGIC FORM 11 ON LITTLE CLUB WHEAT[1]

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INTRODUCTION

Cytological study of plant diseases brings out facts otherwise inaccessible. A study of the behavior of host and parasite in the infection, the details of their interaction as seen under the microscope, is one avenue of approach to the larger field of immunity.

The present problem was suggested by E. B. Mains, who noted that the susceptibility of Kanred wheat to *Puccinia triticina* varied with age and environment, and thought that a microscopical study of the different types of reaction to the rust might help to explain it. The work was soon broadened to include a study of Little Club, a variety uniformly susceptible to the rust, and Malakoff, a strongly resistant variety. This paper presents that part of the investigation dealing with Little Club.

The leaf rust of wheat (*Puccinia triticina* Erikss.) was formerly included with other species under the name of *Puccinia rubigo-vera*. Eriksson (*14*) [3] separated it under the name *Puccinia triticina*. A historical account of the studies of this rust is given by Jackson and Mains (*20*).

The uredinia and telia are found on wheat. Until recently, the aecial host was unknown in spite of numerous attempts to determine it. The work of Jackson and Mains in 1921 (*20*) proved that the aecia are borne on species of Thalictrum native to Europe and Asia, particularly *T. delavayi* and *T. flavum*.

The bearing of this on the origin of the rust is stated as follows (*20, p. 170*): "*Puccinia triticina* is considered to be of foreign origin, because wheat, for which it shows close specialization, is an introduced host, and because the most susceptible species of Thalictrum which serve as aecial hosts also are exotic." In America, where the aecial hosts are practically absent, the rust probably lives on as a continuous series of uredinial generations.

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[2] The writer is indebted to C. R. Ball and H. B. Humphrey for suggestions and for a careful reading of this manuscript, to E. B. Mains for suggestions and materials, and to the Divisions of Genetics and Agronomy of the University of California for courtesies extended during the work.

[3] Reference is made by number (italic) to "Literature cited," p. 221.

MATERIAL AND METHODS

The strain of leaf rust used in these studies was collected by W. W. Mackie at Eureka, Calif., in the summer of 1923. It was cultured in the greenhouse, a single-spore culture was made, an increase generation was grown from the single uredinium, and the rust was carried on in greenhouse cultures. A specimen sent to E. B. Mains was identified as *Puccinia triticina* physiologic form 11.

The seedlings used for cytological study, Little Club C. I. 4066, were grown and inoculated in the field, the first set in April, 1924, the second in May, 1925. They were covered during the 48 hours following inoculation. The older infected plants studied were grown in the field in the late winter and spring of 1925, and were continuously infected from the seedling stage to maturity.

Material was fixed at intervals from the time of inoculation until the uredinium was old. The fixing fluid was chrom-acetic-urea, of the usual strength or diluted. Material fixed in the cold (40° to 45° F.) proved superior to that fixed at room temperature. The usual methods of dehydration and embedding were followed. Paraffin of 50° C. melting point gave the best results The triple stain was most satisfactory.

INVESTIGATIONS

PHENOMENA OF ENTRANCE

The seedlings used in the study of entrance phenomena were from seed sown on March 5, 1925, and had encountered rainy weather. The upper leaves (fourth to sixth) were heavily inoculated May 11 during a rain, and were covered to prevent the washing off of the spores.

Many spores germinated during the first night, others during the second, and still others during the third. Freshly formed germ tubes could be found in any lot of material fixed during the first few days.

Infection by urediniospores takes place through the stomata, which, in wheat, are abundant on both surfaces of the leaf. The germ tube grows along the leaf surface to a stoma. Then the protoplasm along the whole length of this tube flows on into the tip, forming there an aggregation called the appressorium. When unobstructed, it rounds up into a hemispherical cushion of rather dense protoplasm just over one end of the stomatal aperture. A typical appressorium is represented in Plate 1, A. The stoma is cut longitudinally, showing one guard cell *a*, with its unevenly thickened walls and its plastids and elongated dumb-bell-shaped nucleus. The appressorium *b* occupies one end of the shallow cavity at the entrance of the stoma. A portion of the empty germ tube is seen at *c*, and a septum isolates it from the appressorium.

EXPLANATORY LEGEND FOR PLATE 1

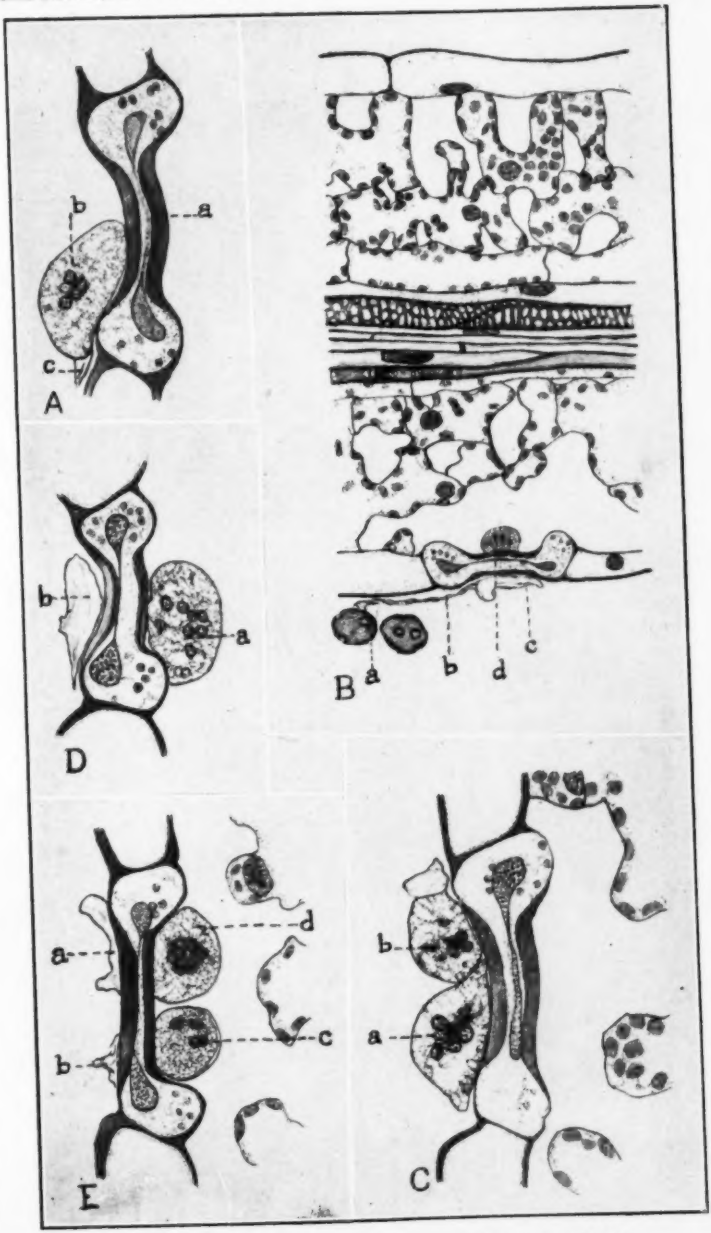
A.—Third day after inoculation. Longitudinal section of stoma bearing appressorium. Guard cell at *a*, remnant of germ tube at *c*, appressorium with 4 nuclei at *b*. × 730

B.—Section through leaf with fungus at stoma. The spores are at *a*, germ tube at *b*, empty appressorium at *c*, and substomatal vesicle at *d*. × 333

C.—Stoma bearing two distinct appressoria, *a* being the older, with *b* partially superposed upon it. × 730

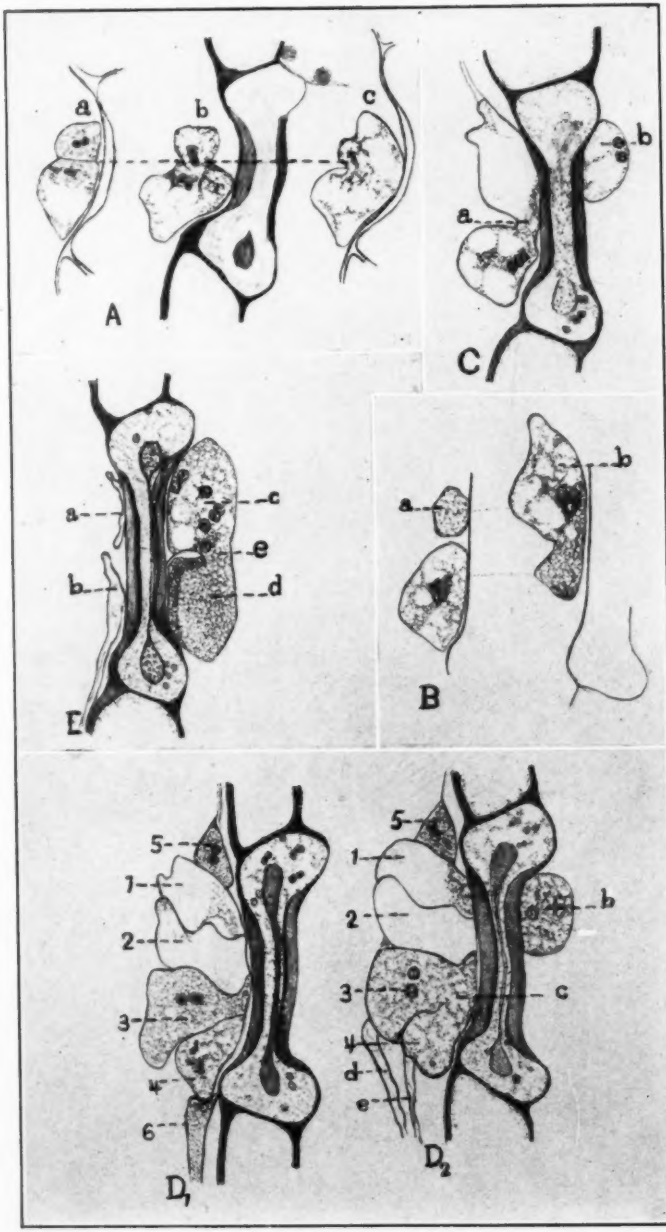
D.—Stoma with the empty appressorium *b* on the outer surface and the substomatal vesicle *a* within. The vesicle contains eight nuclei. × 730

E.—Second day after inoculation. Stoma with two fungi. The appressoria *a* and *b* entered at the ends of the stomatal slit, forming the vesicles *c* and *d* within. One vesicle has four nuclei, the other more. × 730



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 202)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 203)

When, in inoculation, the spores are placed on the upper surface of the leaf, they naturally adhere to the minute parallel ridges running lengthwise on the surface of the leaf. As the longitudinal rows of stomata lie near the base of the little valleys between the ridges on the leaf, the distance the germ tube must grow to its goal may be considerable. The lower surface of the leaf is smoother, and here spores may lodge close to the stoma, and one sometimes sees, as in Plate 1, B, the spore *a*, the germ tube *b*, the appressorium *c*, and the substomatal vesicle *d*, all in the same section.

It is not unusual to find two or more appressoria occupying the same stoma. (Pl. 1, C.) In this case *a* is apparently the older; and *b*, the late comer, is partly superposed on *a*. Fully formed appressoria usually have four nuclei (pl. 1, A and C, at *b*) or sometimes more (pl. 1, C, *a*).

Entrance usually is effected near the end of the stoma. The delicate wall of the appressorium collapses (pl. 1, D, *b*) as its contents pass through the stomatal slit to form the vesicle within. The substomatal vesicle (pl. 1, D, *a*) lies in contact with the inner face of the guard cells. It is an ovoid or irregularly ellipsoid body containing dense cytoplasm and nuclei. When a stoma carries more than one fungus, both may enter. In Plate 1, E, the two collapsed appressoria at *a* and *b* are on the outer surface, and just within are the two separate vesicles, *c* and *d*. The number of nuclei in the vesicle varies. In Plate 1, E, *c*, there are four; in Plate 1, D, *a*, there are eight; in Plate 1, E, *d*, the nuclei form a central cluster so dense that they can not be counted, but there probably are more than eight.

Material was fixed twice a day during the early stages of infection. In the material fixed on the first three mornings, many fungi had just entered the host and formed the substomatal vesicle. (Pl. 1, D and E.) In material fixed in the afternoons the majority of these substomatal vesicles had pushed out the first infecting hypha. (Pl. 3, B.) This daily rhythm in the early stages of infection suggests at least that the time of entrance is conditioned by the daily stomatal movements. It is perhaps indicative that entrance is not effected by mechanical force or by chemical action, but waits upon the natural opening of the stoma. That the fungus, however, does secrete some substance that affects the guard cells seems probable. Its effect is usually very inconspicuous at this stage, but in slightly older infections (pl. 3, C) the guard cell walls are often noticeably altered at the surface of contact with the fungus. Rarely in young infections, but more commonly in older, the stomata occupied by fungi are killed. The connection, if any, between this action on the guard cells and the entry of the fungus is not clear.

Entrance counts were made of fungi fixed in the morning, 40 hours after inoculation. Only 37 out of 204 (about 18 per cent) had not entered.

EXPLANATORY LEGEND FOR PLATE 2

A. Section of dead guard cell bearing an appressorium. Latter drawn at three levels—*a*, *b*, and *c*. At the upper (*a*) it looks like two in contact; at the median (*b*) and the lower level (*c*) the two are connected. × 730

B.—Successive sections of same stoma. Appressoria appear separate in *a* and connected in *b*. × 730

C.—Two appressoria connected at *a*. Single substomatal vesicle forming at *b*. × 730

D_1 and D_2.—Successive sections of stoma bearing six fungi. Those numbered 1 and 2 are nearly empty and a single vesicle (*b*) is forming within. Those numbered 3 and 4 appear separate in D_1; connected in D_2. They have separate germ tubes, (*d* and *e*). Nos. 5 and 6 (in D_1) are younger, and fill in the ends of the stoma. × 730

E.—Two appressoria formed at *a* and *b* entered, forming vesicles *c* and *d*, which became joined at *e*. × 730

The fungi which failed to enter were not uniformly distributed on the leaf but occurred in restricted areas. Throughout four or five successive slides all the fungi would have entered; in the next, the majority would still be outside. This may be correlated with the occurrence of patches of open and closed stomata in wheat, as noted by Loftfield (*25*).

An abundance of spore inoculum was used, and stomata crowded with fungi were of rather frequent occurrence. In Plate 2, A, the highly irregular mass of fungous plasm outside the stoma is drawn at 3 planes in the same section, *a*, *b*, and *c*. At the upper focus, *a*, it appears as two unequal appressoria in contact. At a slightly lower focus, *b*, the two are connected. Still lower, at *c*, the connection is broader. No other fungi or débris were near, and there was no apparent external reason for the irregularity in the shape of this mass. The conformation and the size suggest an incomplete union of two appressoria.

Plate 2, B, represents a similar case. Two successive sections are drawn. In one section, *a*, it appears as two separate bodies; in the other, *b*, the two are connected. The plane of the section is slightly oblique, the bulk of one mass lying in one plane, and the bulk of the other in another. On comparing with Plate 1, A and C, one sees that, in size, it is equivalent to two appressoria.

In Plate 2, C, the two are connected by a narrow bridge, at *a*. In this case entry is in progress, as indicated by a single substomatal vesicle forming at *b*.

In Plate 2, D_1 and D_2, the situation is more complex. Two successive sections are drawn. No fewer than six fungi are present at this stoma. The oldest, numbered 1 and 2, are nearly emptied. No connection between the two was detected, but only one vesicle (D_2, *b*) is forming inside. The appressoria numbered 3, and 4 appeared separate in D_1 and connected in D_2. The empty germ tubes leading into them at D_2, *d* and *e*, allow no question as to the separate origin of the two. Nos. 5 and 6 appear to be younger, and fill in the two ends of the stoma.

In a few cases, as that in Plate 2, E, the two appressoria *a* and *b* remain distinct, effect separate entries, and then the substomatal vesicles *c* and *d* become connected. Another case (pl. 3, A) is an oblique section of both stoma and fungi and is included only because of the surprising similarity of contour and mode of joining, to these features figured in Plate 2, E. These are all drawn to the same scale. On comparing the substomatal vesicles of Plate 2, E, with those in Plate 1, E, one sees that the volume of the united vesicles in Plate 2, E, is at least equal to the total volume of the two separate vesicles in Plate 1, E.

EXPLANATORY LEGEND FOR PLATE 3

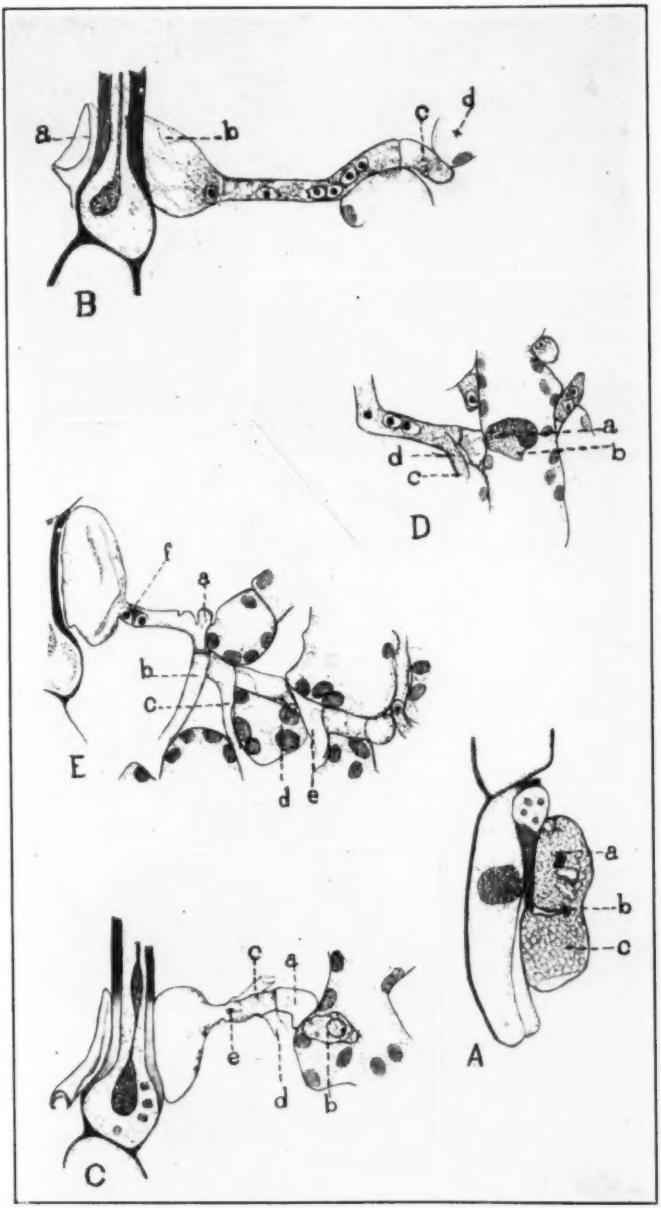
A.—Second day after inoculation. Lateral section of stoma and two substomatal vesicles (*a* and *c*), the latter joined at *b*. × 730

B.—Fungus making first attack. Appressorium at *a*. The substomatal vesicle has formed an infecting hypha with haustorium mother cell *c*. The hypha contains six nuclei. A small haustorium appears in the next section at a point corresponding to *d*. × 730

C.—Six-day infection. The first haustorium (*b*) formed from the haustorium mother cell *a*. Host cell apparently is not injured. The infecting hypha gave rise to branches *c* and *d*. One nucleus (*e*) remains in hypha. × 730

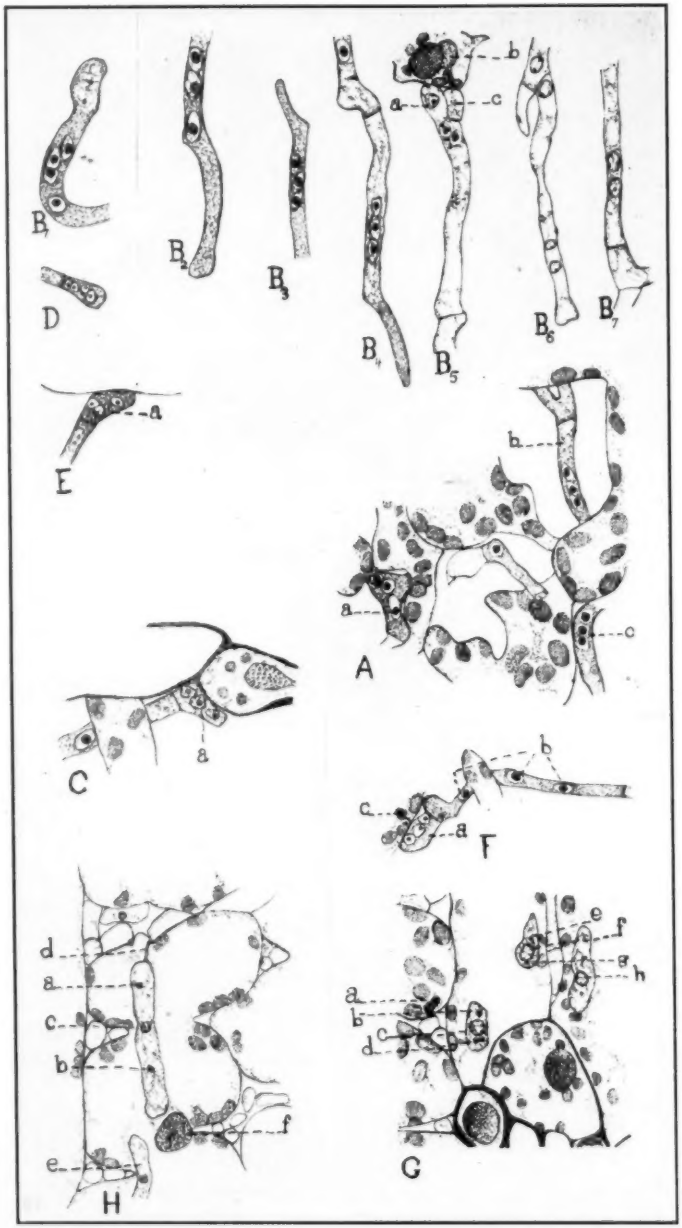
D.—Three-day infection. The first infecting hypha formed a haustorium from the mother cell *d*, and then branched at *c*. The haustorium *b* is partly surrounded by the host nucleus *a*. × 730

E.—Six-day infection. The infecting hypha has formed branches at *b*, *c*, *d*, and *e*. Two nuclei (*f*) remain near the base of the vesicle. × 730



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 204)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 205)

An attempt has been made to determine whether fusions of germ tubes take place before the formation of appressoria. Spores were germinated on glass slides coated with a thin film of vaseline. The vaseline served the double purpose of stimulating germination and fastening the spores and germ tubes to the slide. Permanent preparations were made. The material was fixed by immersing the slide in fixing fluid, then washed, dehydrated, stained, and mounted in balsam. A rich growth of germ tubes was obtained, but no indication of fusion was noted.

VEGETATIVE GROWTH OF THE FUNGUS

After entry the fungous plasm originally present in the spore is massed in a rounded vesicle just inside the stoma, and this move has been accomplished without food other than that originally stored in the spore. The next step is the formation of the infecting hypha. The hypha grows inward across the air chamber beneath the stoma, taking a course at right angles to the epidermis of the leaf. (Pl. 3, B, C, E.) This is in marked contrast to the behavior of the first hypha in stem rust (*2*), which skims along the inner surface of the epidermis to the mesophyll cell nearest the end of the stoma.

Cytoplasm and nuclei flow into this infecting hypha (pl. 2, B), leaving the vesicle *b* nearly or quite evacuated. On striking a mesophyll cell (pl. 2, C) a septum may form just back of the tip, giving rise to a short terminal cell, *a*, the haustorium mother cell. The hypha may pass the first cell, however, and not start this process until the second contact. (Pl. 2, B.) In a number of young hyphae, as in Plate 2, B, six nuclei were counted. The nuclear content of the first haustorium mother cell is not easy to determine, because it usually is dense; but, as the vesicle often contains eight and the hypha six nuclei, the haustorium mother cell probably has two.

In Plate 3, B, a dense young haustorium has formed and appears in the next section at a point corresponding to *d*. In the older specimen drawn in Plate 3, C (fixed six days after inoculation), the mother cell *a* is empty and the haustorium *b* has expanded and its nucleus shows the chromatin network and the dense peripheral granule of the typical haustorial nucleus.

In no case observed, up to the sixth day at least, has the host cell suffered visibly from the invasion. It is not plasmolyzed, collapsed, nor even impoverished. The only disturbance noted is in the host nucleus, which may move over to the haustorium. This would appear to be an independent motion of the nucleus, unaccompanied

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EXPLANATORY LEGEND FOR PLATE 4

A.—Portion of vegetative mycelium in 6-day infection, showing groups of three nuclei in the hyphae at *a*, *b*, and *c*

B_1 to B_7.—Single hyphae showing nuclear content. B_1 hypha from 5-day infection; cel)multinucleate. B_2 and B_3, from 6-day infection, growing tips with three nuclei. B_4, trinucleate cell from 8-day infection. B_5 and B_6, vegetative trinucleate cells from margin of 16-day infection. B_7, pair of nuclei in cell just below epidermis beyond the edge of the uredinium. × 730

C.—Haustorium mother cell (*a*) with three nuclei. × 730

D.—Haustorium mother cell with four nuclei from 5-day infection. × 730

E.—Haustorium mother cell with three nuclei from 12-day infection. × 730

F.—Detail from 5-day infection. Three nuclei in hypha, *b*. Trinucleate mother cell at *a*, young haustorium at *c*. × 730

G.—Nine-day infection. Very young haustorium at *a*. Haustorium at *c*, with one spherical nucleus and granules at *b* and *d*. Haustorium at *f* with granules at *e* and *g*. In the older haustorium (at *h*) the two granules have disappeared. × 730

H.—Large haustorium with one nucleus (*c*) and granules at *a* and *b*. Host nucleus (*f*) midway between haustoria *c* and *e*. × 730

by the other cell contents; at least no massing of cytoplasm and plastids about the haustorium has been noted. In two cases observed, one of which is illustrated in Plate 3, D, the host nucleus *a* is wrapped around the young haustorium *b*, partly inclosing it.

In all cases the infecting hypha gives rise to one or more branches soon after its own advance is checked by the formation of a haustorium. (Pl. 3, C, *c*, *d*; pl. 3, D, *c*; and pl. 3, E, *a*, *b*, *c*.) These branches grow, come in contact with host cells, form haustorium mother cells and haustoria, and in their turn they branch, and repetition of this process soon results in a rich branching system of hyphae. Early in this development the older hyphae are partly or wholly empty, their contents apparently having drained toward the growing tips. This conservation of living material speeds the advance of the fungus.

The nuclear history during this vegetative development has been followed in some detail. Uredinial generations of rusts are considered to be sporophytic, and as a rule are made up of binucleated cells. The physiologic form of leaf rust studied here presents considerable deviation from this rule.

As already noted, the spore has two nuclei, the appressorium four or more, the substomatal vesicle commonly eight, and the infecting hypha, after forming the first haustorium mother cell, often contains six. In the further development, one or two of these nuclei usually are left behind close to the substomatal vesicle (pl. 3, E, *f*; and pl. 3, C, *e*), and the others divide and their progeny become distributed to the branches. Early hyphae of the young fungus have a somewhat irregular nuclear content. Four or even more nuclei in a cell (pl. 4, B_1) are not rare. Soon, however, groups of 3 nuclei (pl. 4, A, *a*, *b*, *c*; and pl. 4, B_2 to B_6) become conspicuous.

Hundreds of these groups of three nuclei have been encountered in infections of different ages, grown at different times of the year and on different hosts. It is not a simple matter, however, to determine that vegetative cells are regularly trinucleate. The hyphae are confined to the irregular communicating air spaces of the spongy mesophyll tissue, and it is seldom possible in sectioned material to trace an individual hypha through any great distance. Moreover, the septa delimiting a cell are thin, and in young hyphae with dense cytoplasm they are easily overlooked. When, occasionally, cell limits are clear, especially in older, partly drained hyphae (pl. 4, B, B_5, B_6), it is evident that trinucleate cells are being dealt with. It is not obvious why the number should be three.

This trinucleate condition in a mycelium does not originate in connection with a fusion of two fungi at the beginning of infection. Young fungi occur with characteristic groups of three nuclei, in which it can still be determined with certainty that a single appressorium was present at the point of entry.

Haustoria form and expand freely. They are regularly uninucleate. In view of the multinuclear mycelium giving rise to them, it was of interest to trace their origin and growth. When the tip of a hypha strikes against a host cell, and its growth in length is forcibly checked for the moment, the changes preparatory to haustorium formation set in. The nuclei divide, one set of daughter nuclei moves out into the thickened tip of the hypha, and a cross wall formed just back of this tip isolates a short terminal cell, the haustorium mother cell. This cell usually flattens out somewhat against

the host-cell wall and often becomes shoe-shaped. (Pl. 4, C, *a*; pl. 3, C, *a*; pl. 3, D, *d*; and pl. 4, E.) It regularly has three nuclei (pl. 4, C, *a*; pl. 4, F, *a*; and pl. 4, E, *a*); rarely two or four (pl. 4, D). These nuclei become markedly reduced in size, as may be seen by comparing them in Plate 4, F, at *a*, with their sister nuclei in the hypha at *b*.

The contents of the haustorium mother cell now pass through the wall into the host cell. First a slender tube forms, extending into the host cell, usually at right angles to the wall at that point. The cytoplasm and the three minute nuclei of the mother cell pass through this tube or "neck" of the haustorium, forming at its inner end a dense globular head within which no details of structure can be seen at first. (Pl. 4, F at *c* and G at *a*.) By absorption of water, it soon expands and the cytoplasm of the haustorium opens into a reticulate structure within which appears a single globular nucleus. (Pl. 3, C, *b;* pl. 4, G, *c, f, h;* and pl. 5, A.) This nucleus contains a rather coarse chromatin net and a single densely stained rounded granule at its periphery.

The question at once arises as to what becomes of the other two nuclei. None remains behind in the mother cell. Do all three fuse to form the single nucleus found in the haustorium, or do two of the three degenerate? A study of half-grown haustoria shows the regular presence of two small dense bodies in the cytoplasm, usually near the nucleus and on opposite sides of it. In Plate 4, G, the haustorial nucleus *c* is attended by small masses at *b* and *d*, and the nucleus at *f* by bodies at *e* and *g*. Sometimes these appear fragmented, as in Plate 5, A, *b, c*. Ordinarily they disappear as the haustorium matures (pl. 5, E, F) but may occasionally still be seen (pl. 4, H, *a, b*). It seems probable, then, that the central nucleus survives and that the other two nuclei degenerate.

Haustoria extend in all directions within the cell, and it often happens that the neck of the haustorium is removed in sectioning. It is assumed, however, that in life a haustorium continues to be connected by this tube with the mother cell outside. Ordinarily the neck remains slender. (Pl. 4, H, *d*; and pl. 5, E, *a*.) A few cases have been seen, as in Plate 5, F, at *a*, in which the neck shortens and thickens and the host wall near by swells.

In shape the great majority of the haustoria are cylindrical or worm-shaped. A few (pl. 5, E, *b*) become irregularly branched. They attain large size. In 9-day infections the 10 largest that were measured averaged 39 μ in length, and in 16-day infections they averaged 44 μ.

The nucleus of the host seems to be powerfully attracted by the haustorium. A nucleus is often seen flattened out against it. (Pl. 5, B, *a, b*.) When two haustoria lie a short distance apart (pl. 4, H, *c, e*) the nucleus *f* is often midway between the two. When nearer together (pl. 5, D, *a, b*) the nucleus may be drawn out into a dumb-bell-shaped body (*c*) having contact with both haustoria. Its shape suggests amoeboid motion, but of course in fixed and stained material the evidence is inadequate. An unusual case is seen in Plate 5, C. The host cell is binucleate, and both nuclei (*a, b*) are stretched out against the haustorium, *c*. This contact of nucleus and haustorium does no visible harm to either body; both continue to live and function.

Of rare occurrence is the peculiarly degenerated haustorium figured in Plate 5, G, *a*. It gains significance from the fact that these occur commonly in infections of this rust on less susceptible hosts. Even this dead haustorium is accompanied by the host nucleus, *b*.

REPRODUCTION

Reproductive activity begins early in leaf rust. The first spores are liberated on the eighth or ninth day, or sometimes as early as the seventh. With the onset of reproduction there comes a marked change in nuclear relations. Any section through the margin of a uredinium where the spores are still young and thin-walled (pl. 6, A) shows that spores are uniformly binucleate. A small portion of spore-bearing tissue is drawn on a larger scale in Plate 7, A. Spores (pl. 7, A, *a*, *b*) and stalk cells (*c*, *d*) are binucleate. The reproductive cells beneath are partly drained, the cytoplasm is diminished in amount, and the nuclei are reduced in size and sometimes indistinguishable. Occasionally, however, as in Plate 7, A, *e* and *f*, cells at some depth below the surface are binucleate.

Feeding hyphae at the center (pl. 6, A, B) are too closely interwoven to make it possible to determine cell limits. Moreover, the translocation of food materials from the central mycelium to the reproductive areas at the surface has left these hyphae nearly empty, and their nuclei, when visible at all, are vague.

It is in the marginal regions of the infections, if anywhere, that the mode of origin of the binucleate condition is to be determined. Apparently it is in only those cells soon to give rise to spores that this change takes place, for vegetative hyphae of the deeper tissues still show the groups of three nuclei. (Pl. 4, B_5.) Even a week or 10 days after reproductive activities have begun, one still may see them in feeding hyphae in the outskirts of the infection. In B_5 in Plate 4, taken from a 16-day infection, is a young haustorium (*b*) from a mother cell (*c*), and just below it the three nuclei are passing out into the branch at *a*.

But along the line of spore formation just below the epidermis, fungous cells become binucleate. As the uredinium spreads radially, more and more of the subepidermal mycelium changes character, and this takes place considerably in advance of spore formation. Subepidermal binucleate cells may sometimes be found 150 μ beyond the young spores.

Attempts to discover just how this change takes place have led to rather puzzling results. Theoretically it might happen in any one of several ways. A trinucleate cell might divide into two, leaving one nucleus behind in the penultimate cell and two in the terminal cell. In its further growth the terminal cell would give rise to a series of binucleated cells. This appears to happen in some cases. In Plate

EXPLANATORY LEGEND FOR PLATE 5

A.—Haustorium with nucleus at *a*, and a group of small granules at *b* and *c*. Host wall swollen and disintegrated at *d*. × 730

B.—Haustorium (*a*) with host nucleus (*b*) flattened against it. × 730

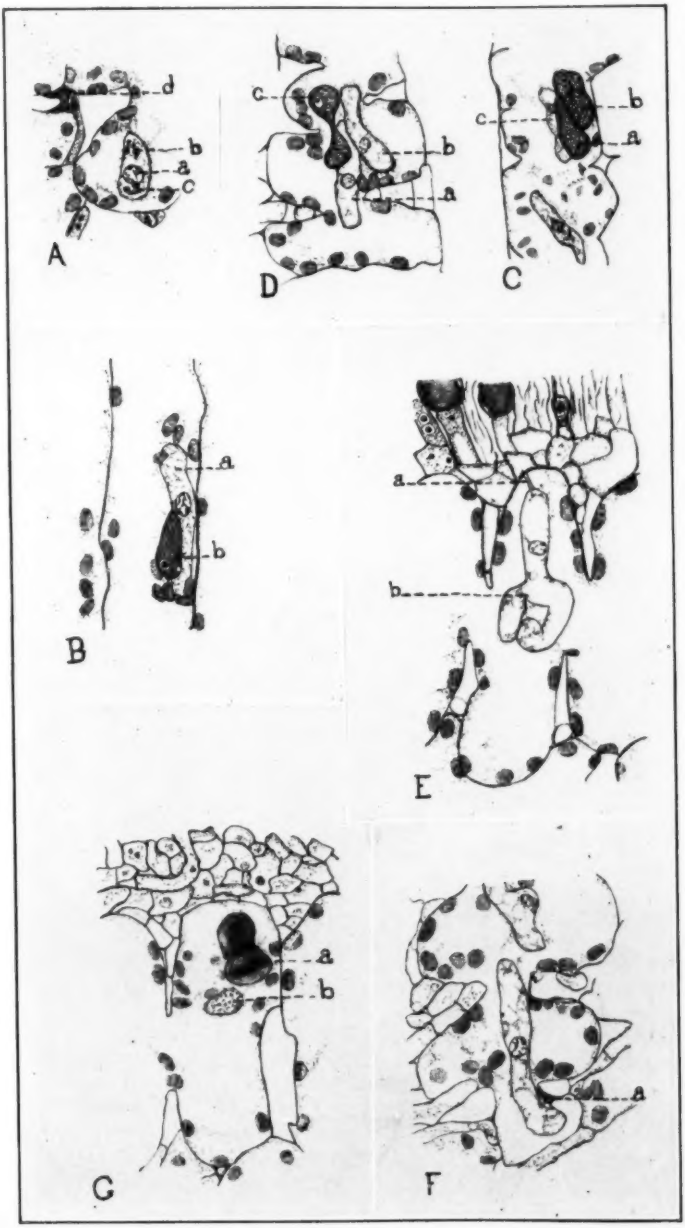
C.—Host cell binucleate, and both nuclei (*a* and *b*) are stretched out along the haustorium *c*. × 730

D.—Dumb-bell-shaped host nucleus *c* making contact with two haustoria, *a* and *b*. × 730

E.—Branched haustorium (*b*) with long, slender neck (*a*), in 16-day infection. Host cell still vigorous. × 730

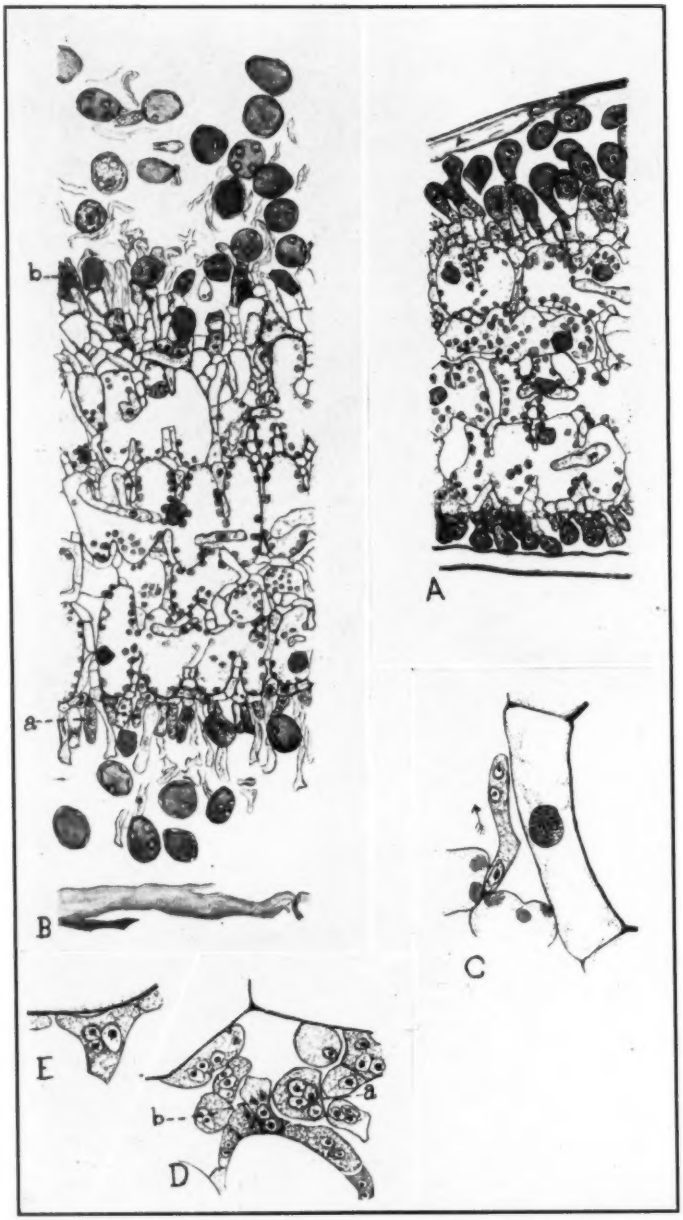
F.—Old haustorium with short, thick neck at *a*, and the near-by host cell wall swollen. × 730

G.—Detail from 16-day infection. Dead haustorium *a* composed of central core, and a lighter-staining outer layer. Host nucleus at *b*. × 730



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 208)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 209)

7, C, there are binucleate cells (*b*, *c*), and back of them, in the same hypha, is a uninucleate cell (*a*).

The binuclear condition also would be established if one nucle s of a trinucleate cell divided, forming a cell with four nuclei, and a cross wall separated it into two cells of two nuclei each. No direct proof of this has been seen. Subepidermal cells containing four nuclei occur. (Pl. 6, D, *a*.) If binucleate cells arose in this manner it would be difficult to prove it after the event, for the result of an ordinary division of a binucleate cell would look the same.

A binucleate cell might arise by the degeneration of one of the three nuclei, by some process similar to that which regularly occurs in the haustorium. This also is not proved, although such cases as the cell in Plate 6, D, *b*, with two normal nuclei and dense granules near by, are suggestive.

Two nuclei of a trinucleate cell might fuse, giving rise to a binucleate cell, but there is no evidence that this occurs. The nuclei of these short cells are usually in contact, and occasionally one sees a large nucleus with two nucleoli, but this in itself is not proof.

In Plate 6, C and E, are two cells each containing one full-grown nucleus and a pair of half-grown nuclei. This condition probably arose by the independent division of one of the nuclei of a binucleate cell. Evidently the simultaneous "conjugate divisions" which maintain the binucleate condition in other rusts are not well established in this readjustment period.

Possibilities of a different type are indicated in the specimens drawn in Plate 7, D and E, one of which (D) was found in a 6-day infection and the other (E) in a 12-day infection. In both of these, two hyphae converged and fused (pl. 7, D and E, at *a*) and an enlarged mass formed at the point of union. In Plate 7, D, the two fusing hyphae (*b* and *c*) can be traced back a considerable distance. One, at least (*b*), is multinucleate. Coming out of the fused mass are one abortive hypha (*d*) and one vigorous hypha which has formed a haustorium mother cell, at *e*. A small haustorium from it appears in the next section, and a branch (*f*) contains a pair of nuclei. Plate 7, E, is similar in essentials. The mycelium near by is mainly binucleate.

This process suggests at once the fusions of two separate fungi noted at the stoma at the beginning of infection. The question arises as to the parentage of the fusing hyphae here. When adjoining stomata are entered by individual sporelings, their resultant mycelia become intimately intermingled. Under such circumstances adjoining hyphae might come from different parents.

In neither of these cases is the evidence absolute. In the section, a portion of which is figured in Plate 7, D, there are several young infections slightly overlapping. The hyphae that fuse can be traced back toward the point of entry. The point *g* is only 50 μ away from the original infecting hypha at the stoma, and the other

EXPLANATORY LEGEND FOR PLATE 6

A.—Marginal portion of a uredinium in a 9-day infection. Host tissues vigorous. Young spores and stalk cells binucleate. × 333

B.—Portion of section through 16-day infection. Fungus not massive, but spore production heavy at both surfaces. Host tissues living and not seriously impoverished. × 333

C.—Fungous cell just below epidermis, 200 μ beyond margin of uredinium. Contains a pair of young nuclei at the tip and one large nucleus at the base. × 730

D.—Subepidermal mycelium in the region of transition to binucleate condition, containing two to four nuclei per cell. Four nuclei in cell at *a*. At *b*, cell with two nuclei and two dense granules. × 730

E.—Cell just below epidermis, with pair of small daughter nuclei and one full-grown nucleus. × 730

hypha (*c*) came from the same direction. The probability is that in this case the fusing hyphae are rather closely related. In an older infection this would be still harder to determine, for the early history of the fungus is more or less obliterated by the later growth. In the older infection from which Plate 7, E, was drawn, only one discolored stoma was noted (indicative of the point of entry of the fungus), but, as part of the epidermis over the open uredinium was missing, it is possible that more than one mycelium was present.

How frequently and under what conditions this type of fusion occurs is not known. Both of these cases were found in the relatively free space of a substomatal cavity. In almost any other location they would pass unnoticed. Even here they would have been overlooked after other hyphae had closed in around them.

Taking place as it does in tissues about to form spores, this fusion also is vaguely suggestive of the fusions which occur at the base of the aecium in other rusts. Nothing is known of the nuclear phenomena here, nor of the later development from the fusion, nor what share, if any, the fusion has in the establishment of the binucleate spore-bearing tissue.

By whatever process or processes the shift to the binucleate condition takes place, it works efficiently. The mother cell of the spore (pl. 7, A, *g*) is regularly binucleate, and upon its division the spore and the stalk cell each receive 2 nuclei. (Pl. 7, A, *a*, *b*, *c*, *d*.)

As the spores mature they round up, their wall thickens, and their stalks elongate. When the stain is favorable, a number of scattered germ-pores in the spore wall can be seen. (Pl. 6, B.) The spores are set free by the rupture of the epidermis. The spore stalks wither and new spores push out between them. (Pl. 6, B, *a*, *b*.) This process continues as long as food is available.

In spite of the congeniality of parasite and host, the leaf-rust mycelium in seedling leaves does not attain a very massive growth nor any great diameter. The limited spread of *Puccinia triticina* within the leaf may be explained by the weak development of "runners" or "stolons." Even in 16-day infections the marginal growth of the fungus is composed of ordinary feeding hyphae with haustoria at frequent intervals. The long, straight, rapidly growing, sparsely septate and sparsely branched stolons, so common in stem-rust infections from the sixth day on, are rarely encountered here. Secondary uredinia do occur, however, under favorable conditions. Plate 8, A, is reproduced from a photograph of a 25-day culture on Trumbull, a very susceptible variety of wheat. The circle of secondary uredinia is separated by a narrow clear space from the primary. No second circle of uredinia has been noted. A comparison of this with the corresponding development of stem rust under favorable conditions shows a marked contrast. In Plate 8, B, is shown, at

EXPLANATORY LEGEND FOR PLATE 7

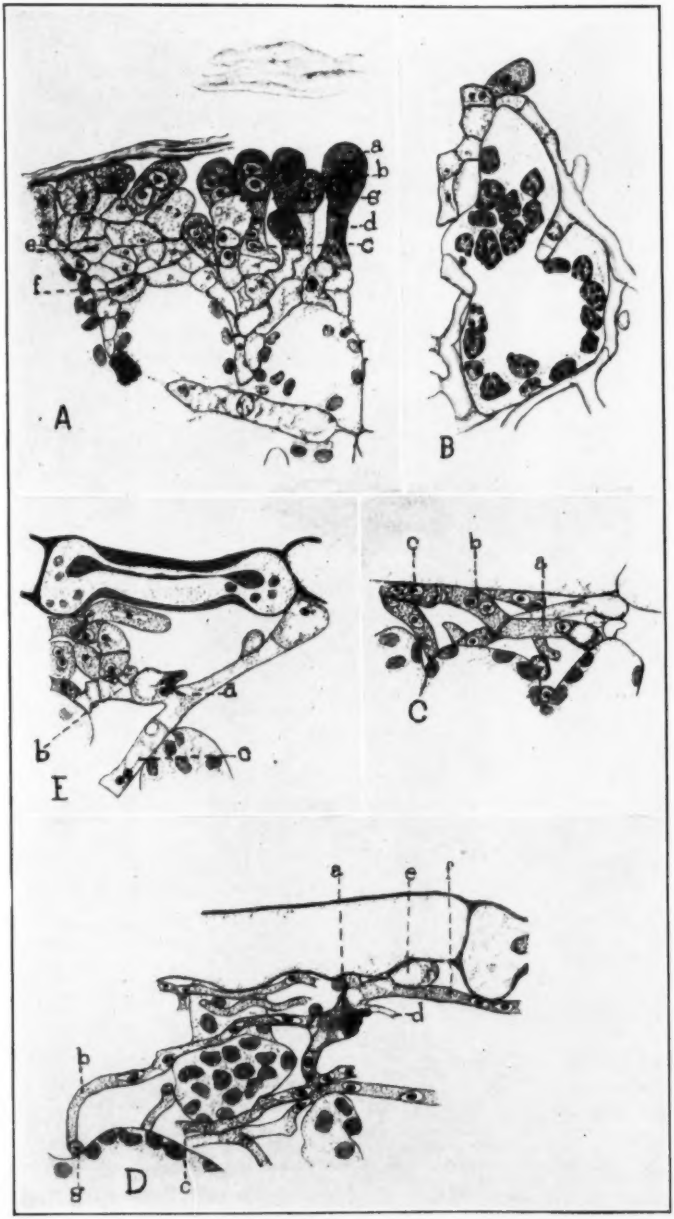
A.—Margin of uredinium from 9-day infection fixed at 4 p. m. Spores (*a*, *b*), stalk cells (*c*, *d*), and deeper lying cells (*e*, *f*) binucleate. Starch content of host tissue moderate. × 730

B.—Cell just below the uredinium of a 10-day infection on a weakened leaf. Plastids with maximum starch content. × 730

C.—Subepidermal mycelium near margin of uredinium. Hypha with uninucleate cell at *a*, and two binucleate cells at *b* and *c*. × 730

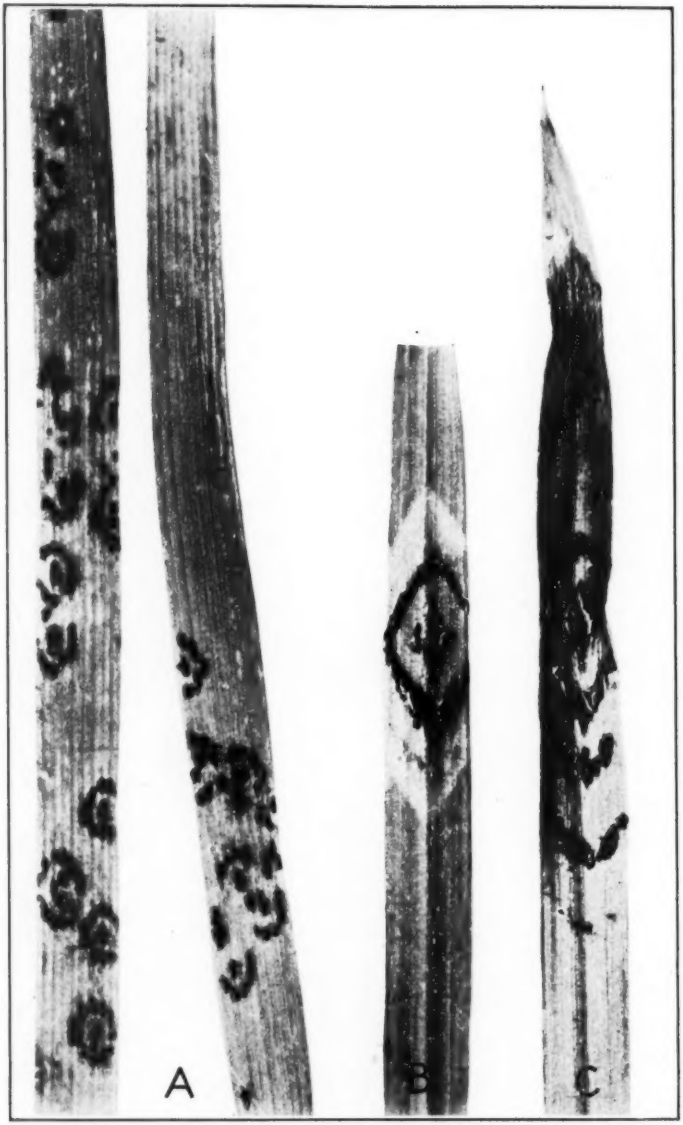
D.—Portion of subepidermal mycelium from 6-day infection near stoma of entrance. Cells chiefly trinucleate. Hyphae *b* and *c* meet and join at *a*. Proceeding from *a* are a vegetative hypha with haustorim mother cell at *e* and branch at *f*, and an aborted hypha at *d*. × 730

E.—Mycelium in substomatal cavity at margin of 12-day uredinium. Cells chiefly binucleate. Cells at *b* and *c* fuse at *a*. From the point of union proceed an aborted tip and a vigorous vegetative hypha. × 730



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

(For explanatory legend see p. 210)



Puccinia triticina physiologic form 11 on seedlings of Little Club wheat

A.—*Puccinia triticina* physiologic form 11 on Trumbull wheat grown in greenhouse. Rust 25 days old. Scant development of secondary sori. × 3.

B.—A single-spore, 23-day culture of *Puccinia graminis tritici* physiologic form 3 on Little Club wheat, showing secondary uredinia. × 3.

C.—Same culture as in B, at 39 days of age, showing further development of secondary uredinia. × 3.

the same magnification, a single-spore infection of stem rust (*Puccinia graminis tritici* physiologic form 3) 23 days old on Little Club. Plate 8, C, shows the same infection at 39 days. Three or four concentric lines of uredinia have formed at broad uniform intervals. The total spread of the fungus is six or eight times that of leaf rust.

RELATION TO THE HOST

The host tissues in and about the infection show remarkably little disturbance in either cell walls or cell contents. Except for the modification of guard-cell walls at the point of entry of the fungus, there is very little effect on the cell walls of Little Club. As far as has been observed, it seems to make very little difference whether the host is young or old, or whether the fungus is grown under favorable or unfavorable conditions; the walls of the host plant rarely are affected. Once in a while a limited spot is swollen (pl. 5, A, *d*), but this is exceptional.

Another phase of the interaction of host and parasite is the heightened osmotic pressure of infected tissue. In all but the youngest infections a fixing fluid which will fix the fungus and the infected host tissues well, often will shrink the healthy tissues of the leaf. It is not clear whether this increased turgor is due directly to some substance secreted by the fungus into the host cell or to the heightened or altered activities of the cell in response to the fungus or to a combination of the two.

The infected tissues live on. Sometimes there is not a single dead cell in 9-day and 12-day infections, and even in 16-day infections there is not more than 1 per cent of dead cells. In former stem-rust studies it was noted that even in fairly congenial relationships the first host cell attacked by the young fungus often rapidly collapsed and died. Here, on the contrary, the cell containing the first haustorium is alive and apparently functioning normally on the fifth or sixth day of the infection. Later than this it is not always possible to identify the first cell, for the early history often is obscured by the massing of the fungus below the epidermis for reproduction. The one or two dead cells in old infections are at the center of the infection and just below the epidermis, however, and may well be the first cells invaded by the fungus.

A closer study of the conditions in infected host cells shows even more clearly how slight are the visible alterations in cell activities. The size of plastids, except when they are gorged with starch, is an index of the vigor of the cell, for in impoverished tissues plastids become reduced and disappear.

Plastids were measured in infections of different ages (Table 1), at the center and the margin of the infection, 50 μ beyond the outermost hypha, and at a distance far enough away to be beyond any influence of the fungus. Each figure in the table represents the average of 20 plastids, 5 from each of 4 infections. Only plastids in the first layer of cells below the epidermis were measured.

The data in Table 1 indicate that there is very little impoverishment of the host tissues in and about the infections. In the 5-day material the normal plastid is 4.4×2.3 μ, and at the center of the infection it is 3.9×2.5 μ. In the 16-day material the normal plastid is 4×2.2 μ, and at the center it is 3.5×2.3 μ. Allowing for the normal

decrease of plastid size in the older leaves, the reduction in size in the older infections is relatively little greater, if any, than in the younger ones. There is a scarcely perceptible impoverishment of tissues beyond the fungus.

TABLE 1.—*The size of plastids in and around infections of Puccinia triticina physiologic form 11 of different ages on Little Club wheat. (Each figure represents the average size in μ of 20 plastids, 5 from each of 4 infections)*

Age of infection	Size of plastids			
	In center of infection	In margin of infection	50 μ beyond margin	Normal
5 days	3.9×2.5	3.9×2.4	4.4×2.3	4.4×2.3
9 days	4.0×2.5	3.9×2.3	4.0×2.2	4.2×2.2
12 days	3.7×2.5	3.6×2.5	4.1×2.7	4.2×2.5
16 days	3.5×2.3	3.6×2.1	3.8×2.2	4.0×2.2

The starch content of these plastids is of interest. A comparison was made of infections of different ages in two sets of seedlings grown under different conditions. (Table 2.) The first set was grown in the field in the spring of 1924 in clear, dry weather; the second, third, and fourth leaves were inoculated; the infected leaves were turning yellow at the end of the series. The second set was grown a year later in a wet season with intermittent dark days; the fourth to the sixth leaves were inoculated; the infected plants grew vigorously, kept their color, and a heavy crop of uredinia developed. In both sets, material was fixed at intervals during the growth of the fungi, and slides were prepared. The range of starch content in each lot was estimated. In grading it, S_0 signifies no visible starch, S_3 the maximum, and S_1 and S_2 intermediate quantities. Each figure in the table is the result of observations on three to six infections.

The contrast between the two sets is marked. (Table 2.) The second set of plants grew well in spite of the heavy infection, and the starch content of the plastids within the infected areas was nearly equal to that beyond them. With one exception, the leaves were fixed in the morning, when normally there is little starch left in the leaf. Where there was a difference between the starch content of the healthy and the infected tissues, the infected tissue contained the most, but the difference was slight. The 9-day material was fixed in the afternoon. The healthy tissue was slightly higher in starch; the infected tissue contained about the same amount of starch as when fixed in the morning.

On the contrary, there was a markedly greater amount of starch in the infected tissue, in the 1924 series. (Table 2, set 1, also fixed in the morning.) The starch content increased up to the tenth day, when limited areas in the center of the infection showed the maximum and the plastids were gorged and swollen with starch. (Pl. 7, B.) The contrast between this and the condition on the ninth day in the 1925 set (pl. 7, A) is striking. The leaf fixed on the fourteenth day was beginning to turn yellow, and plastids outside of the infections were smaller. At the center of the infection the starch was about gone, but near the margin it graded S_2. The situation was substantially the same on the seventeenth day.

TABLE 2.—*Summary of starch content of plastids in two sets of infections of Puccinia triticina physiologic form 11 on Little Club grown in the field, graded from S_0 (no visible starch) to S_3 (the maximum)*

Set 1 Sown Apr. 7, 1924; inoculated Apr. 30, 1924 (dry season)			Set 2 Sown Mar. 5, 1925; inoculated May 11, 1925 (wet season)		
Age of infection	Starch in plastids in infected tissue	Starch in plastids in uninfected tissue	Age of infection	Starch in plastids in infected tissue	Starch in plastids in uninfected tissue
6 days	S_1– to S_2–	S_0 to S_1	5 days	S_{0+} to S_2	S_1 to S_{1+}.
8 days	S_1– to S_2	S_{0+} to S_1–	9 days at 4 p. m.	S_{0+} to S_2–	S_{0-} to S_2–.
10 days	S_2 to S^2. Plastids enlarged.	S_{0+} to S_1–	12 days	S_1– to S_2	S_1– to S_1.
14 days	S_0 at center of infection to S_2 at its margin.	S_{0+} to S_1–. Leaf turning yellow. Plastids reduced in size.	16 days	S_0 to S_1–	S_0 to S_{1-}.
17 days	S_{0+} at center to S_2 at the margin.	S_0 to S_1			

Further information concerning conditions in infections is to be gained by study of the nuclei. Nuclei were measured at the center of infections of different ages and in tissues of the same leaf far enough removed from the fungus not to be influenced by it. The data are given in Table 3. Each result represents the average of 20 nuclei, 5 from each of 4 infections. The normal nuclei vary a little in size. The writer has observed that the nuclei of the cells which contain haustoria are always a little larger than the corresponding nuclei of healthy tissues, and different in shape. The maximum occurs in 12-day infections where the normal nucleus is 8.5×6.2 μ, and the nucleus in diseased tissue is 10.6×6.3 μ. The increase in volume is not so great as the dimensions indicate, however. There is a tendency for the nucleus to stretch out toward or along a haustorium. The diameter of the body of the nucleus remains unchanged, and the long tapering point at one end represents a relatively slight increase in volume. Not all nuclei undergo this elongation, but, taking them as they come, they decidedly affect the average length of the nuclei. This is in marked contrast to a number of cases in stem rust of wheat, in which nuclei increased in volume several fold and then collapsed and died.

TABLE 3.—*Size (in μ) of living nuclei in infection and normal tissue of Little Club seedlings infected with Puccinia triticina physiologic form 11. (Each figure represents the average of 20 nuclei, 5 from each of 4 infections)*

Age of infection	Size of nuclei	
	At center of infection	In normal tissue
5 days	8.8×7.0	8.2×7.1
9 days	10.1×6.3	8.6×6.5
12 days	10.6×6.3	8.5×6.2
16 days	9.1×7.4	8.7×7.4
Average	9.6×6.7	8.5×6.8

Relatively few nuclei die, and the number seems to vary somewhat with the vigor of the rust-infected leaf. Counts were made in several infections of 5, 9, 12, and 16 day material. A nucleus was con-

sidered dead when it had shrunk somewhat and stained a homogeneous red in which no chromatin net was discernible. Fewer than 10 per cent of the nuclei had died, even in the oldest infection studied. (Table 4.) In the exceptionally vigorous tissues of the 12-day infections less than 2 per cent of the nuclei died.

TABLE 4.—*Percentage of dead nuclei in infections of Puccinia triticina physiologic form 11 on Little Club seedlings. (Several infections were included in the count for each age)*

Age of infection	Number counted	Number dead	Percentage dead
5 days	150	3	2.0
9 days	530	39	7.3
12 days	698	13	1.9
16 days	1,056	99	9.4

INFECTIONS ON OLDER HOST PLANTS

A brief comparative study was made of this strain of leaf rust on older plants of Little Club. The seedlings were grown in the field and inoculated artificially. In their later growth the rust spread naturally, infecting the new leaves as they grew. All stages of the development of the rust were present, but the exact age of any particular infection was not known. Material was fixed at three stages—(1) when the culms began to push up; (2) when they were half grown; and (3) the uppermost leaf, when the heads were 3 or 4 inches out of the sheath.

A cytological study of these infections showed only minor differences. The later leaves had more substance than the seedling leaves, and the fungus made a heavier, more massive growth on them. The sori were longer, and the spore output of each sorus was greater than on the seedlings.

In the details of the relations of rust and host there were few differences. Here, as before, there was a luxuriant development of haustoria, but the average length of the largest ones seen was 44 μ, about the same as in the seedlings. Here, too, the invaded host cells maintained their vigor and appeared to function almost normally. The starch content of the plastids within the infections was equal to or slightly greater than that in healthy tissue. The host nucleus was in contact with the haustorium. It usually was spherical or nearly so, but may have been drawn out irregularly, especially if two or more haustoria occurred in one cell. In Plate 9, A, are two unusually distorted nuclei. The nucleus *a* came in contact with the haustorium *b*, then extended into a lobe almost disconnected with the main body of the nucleus, which forms contact with a second haustorium (*c*). In an adjoining cell (pl. 9, A) the nucleus *d* is

EXPLANATORY LEGEND FOR PLATE 9

A.—Detail of mycelium. Lobed host nucleus *a* in contact with haustoria *b* and *c*. The much-elongated host nucleus *d* has formed contacts with haustoria at *e*, *f*, and *g*. × 730

B to E.—Stages in the crushing of subepidermal mesophyll below uredinia.

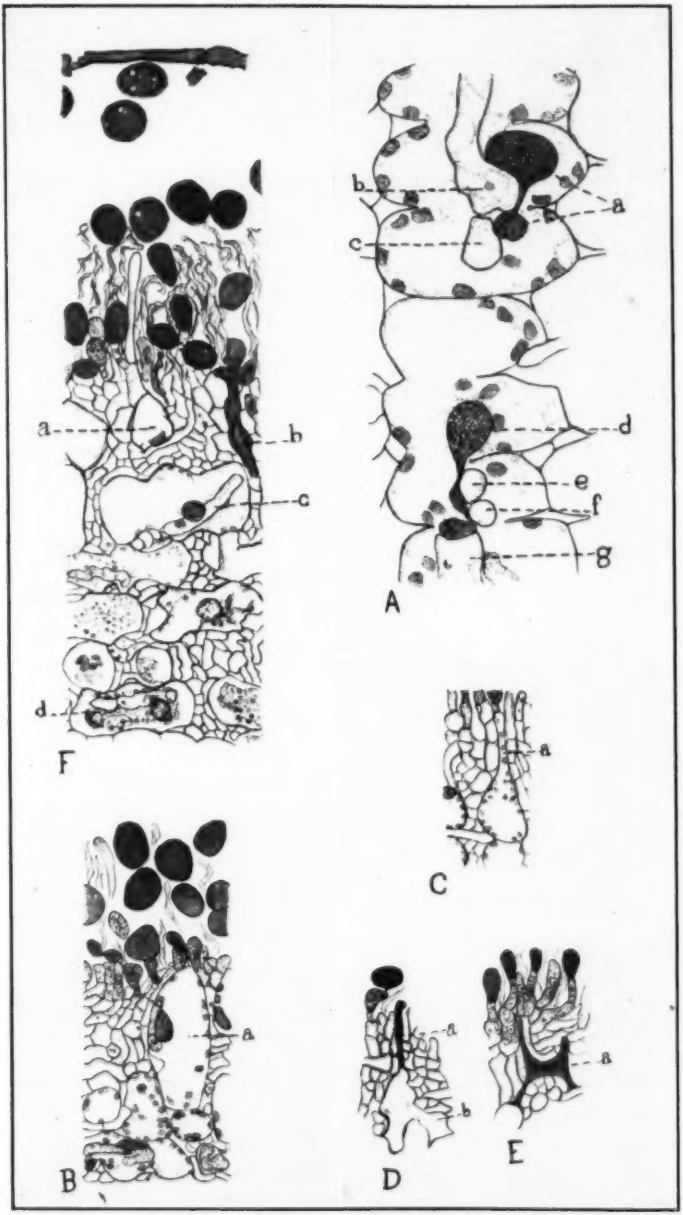
B.—Hypodermal cell *a* somewhat narrowed, due to lateral pressure induced by the mycelium. × 333

C.—Increasing pressure has reduced outer half of mesophyll cell *a* to a mere slit. × 333

D.—Mesophyll cell *b* dead. No cell lumen left in outer half, *a*. × 333

E.—Mesophyll cell *a* completely crushed. × 333

F.—Strip part way through leaf bearing old uredinium. Partly crushed host cell at *a*. Completely killed host cell at *b*. Host cell at *c* still living. Deeper-lying host cells, as at *d*, are not crushed but are plasmolyzed. × 333



Puccinia triticina physiologic form 11 on heading plants of Little Club wheat

(For explanatory legend see p. 214)

stretched out and bent at right angles, forming contacts with haustoria at *e*, *f*, and *g*. It has been commonly believed that haustoria move to the nuclei of the cells they invade. Here, on the contrary, there is little question but that the nuclei move to the haustoria. This activity of the nucleus would seem to be independent of the rest of the cell contents, for there is no marked flow of cytoplasm to the haustoria.

In old infections there are a few degenerate haustoria of the type figured in Plate 5, G.

Not more than 1 or 2 per cent of the host cells collapse and die during the first vigorous development of the fungus, and these are in the middle of the infection. The contents of these cells become dissolved and homogeneous in appearance, the lobes of the cell shrink into slender strands, and the whole cell is reduced to a small irregular mass which stains intensely red in the triple stain.

Later a few cells may die by quite a different process. The mycelium becomes more dense, filling every angle and cranny of the intercellular spaces, and then crushing the cells themselves, deforming them and even obliterating a few of them. This is particularly likely to happen to cells in the layer of mesophyll immediately below the epidermis. Here the richly branching hyphae of the spore-bearing tissue often encroach seriously upon the subepidermal mesophyll. Details of this process are shown in Plate 9, B to F, inclusive. In B the cell *a*, though surrounded by hyphae and somewhat narrowed, is not noticeably harmed. In C the upper half of the cell lumen *a* is greatly reduced, apparently by lateral pressure exerted by the fungus. In D the outer half of the mesophyll cell *a* has shrunk to a discolored strand, and the rest of the cell (*b*) is empty. In Plate 9, E, and F, *b*, the entire cell has collapsed.

One factor which operates mechanically in this process is the swelling of the older drained mycelium. Freshly growing hyphae with dense contents average a little more than 3 μ in diameter. Old empty hyphae, whose contents have drained into the spore-bearing tissue and been used in spore production, increase in size, averaging 5 or 6 μ in diameter. When the threads of a closely interwoven felt of hyphae increase in bulk, the cells they surround are mechanically reduced in volume. Deeper-lying host cells are less frequently destroyed by this process, but if they are completely engulfed by the fungus they become impoverished, and the contents, including haustoria, may become massed at the center of the cell. (Pl. 9, F, *d*.)

These older infections continue to spread marginally, but as far as has been observed, the outermost hyphae are feeding hyphae, not stolons.

On the whole these infections on Little Club form an excellent picture of congeniality between host and parasite. The rust grows freely, and the host lives on with the least possible disturbance compatible with feeding the parasite.

DISCUSSION

Any long-cycle rust shows a regular sequence of nuclear phenomena and a definite alternation of generations. The uninucleate or gametophytic generation begins with the sporidium, continues through the aecial mycelium, the pycnia, and pycniospores. In a layer of cells at the base of the aecium, the transition to the binucleate condi-

tion occurs either by the migration of a nucleus from one cell to another (*4, 5, 28, 24*) or by the resorption of the walls between two adjoining uninucleate cells. (*6, 7, 5, 28, 16, 33, 24.*) The layer of binucleate or sporophytic cells so initiated gives rise by conjugate divisions to binucleate aeciospores. The sporophyte continues on through the uredinial generations. Only in the young teliospores do the two nuclei fuse (*32*). Upon the germination of the teliospore the fusion nucleus divides twice and there is evidence (*32, 19, 4, 27*) that these are reduction divisions. The sporidia borne on the promycelium initiate the gametophyte once more.

Numerous deviations from this regular cycle are recorded in the literature of rust cytology, and to these can be added the minor aberrations found in *Puccinia triticina* physiologic form 11.

Puccinia triticina can continue to live for an extended period by means of an unbroken line of uredinial generations. Indeed, the probability is that it must do so in America, as the aecial host is rarely present. In answer to an inquiry on this point, E. B. Mains states:

> In regard to your question as to the per cent of cases in which the American leaf rust (of wheat) completes its life cycle, I believe that this very seldom occurs. Our work indicates that the only susceptible species of Thalictrum are one or two European species, especially *Thalictrum flavum.*

Teliospores form, to be sure, and germinate (*20*), but in the absence of the aecial host this is a blind alley in the life cycle, and the only effective continuance is by means of urediniospores.

This long-continued uredinial series takes place without apparent diminution in the vigor of the fungus. Cytological study of this physiologic form of the rust, however, reveals a number of irregularities which might be variously interpreted as signs of oncoming degeneration or as sporadic ill-defined ways of compensating for the missing sexual part of the cycle. Briefly, these are (1) the occasional fusion of two appressoria or of two substomatal vesicles; (2) regularly multinucleate cells in the first hyphae, followed by (3) the conspicuous groups of three nuclei and trinucleate cells throughout the vegetative development; (4) regularly uninucleate haustoria from trinucleate haustorium mother cells; (5) occasional fusions of hyphae where spores are soon to be formed; and (6) a complete return to the binucleate condition in the spore-bearing tissue, which then produces normal binucleate spores.

What weight should be given to these variations is a question. No comparative study has been made of this rust passing normally through its complete life cycle. This makes difficult an evaluation of the irregularities found here. A comparison of them with the unusual nuclear behavior found elsewhere in the rusts may be of interest.

Multinucleate cells in the first hyphae of the uredinial mycelium of *Puccinia triticina* were observed by Pole-Evans (*15*) in 1907. He observed them at a similar stage in *P. phleipratensis, P. glumarum, P. dispersa, P. simplex, P. coronifera,* and *P. sorghi.* He seems to regard them as cases of delayed septation. Ward (*34*) figures two to four nuclei in cells of the young uredo mycelium of *P. dispersa.*

Multinucleate cells frequently have been found at other points in the life cycle. Olive (*28, 29, 30*) finds that a large proportion of 50 species studied showed multinucleate cells at the base of the aecium

associated with the process of fusion. Fromme (*17*) finds them at the base of the aecium in *Melampsora lini*.

Lindfors (*24*) finds that the uninucleate mycelium of the primary uredo of *Trachyspora alchemillae*, which spreads throughout the host plant, may become multinucleate in the cramped quarters of the embryonic tissues of the host, but becomes uninucleate again as those tissues expand. He believes that this should be considered as essentially uninucleate. In *Puccinia glumarum* the uredo mycelium is multinucleate, but it becomes binucleate in reproductive areas. He says (*24, p. 28, 30*):

> Nun geschieht auch die Begrenzung der Kernanzahl auf je zwei in jeder Zelle [of the spore-bearing tissue] teils durch Wandbildung, teils wahrscheinlich auch durch Degeneration eines Teils der Kerne. * * * das Myzel, trotz Vorkommens vieler Kerne in einem Zellenleib während der vegetativen Entwicklung, dennoch im Prinzip als zweikernig zu betrachten ist, was aus dem Verhalten der Kerne bei Bildung und Keimung der Uredosporen hervorgeht.

In the cases just cited the nuclei are numerous and indefinite in number and throw little light on the groups of three nuclei found in the later vegetative development of *Puccinia triticina* physiologic form 11.

Trinucleate cells have been figured by a number of students of rust cytology, but are quite uniformly sporadic cases of triple fusion. At the point of transition from the uninucleate to the binucleate condition (at the base of the aecium in the long-cycle rusts, and in the primary uredo or the telium in those of abbreviated cycles), in a few cases three nuclei become associated instead of two. Spores arising from these trinucleate basal cells also are trinucleate. The fate of these spores is unknown. This has been reported by Blackman in 1904 (*4*); Blackman and Fraser, 1906 (*5*); Olive, 1908 (*28*); Dittschlag, 1910 (*10*); Hoffmann, 1911 (*18*); Fromme, 1912 and 1914 (*16, 17*); Kunkel, 1914 (*22*); and Lindfors, 1924 (*24*). These, too, are of little aid in explaining the present case, as the urediniospores giving rise to the mycelium of *Puccinia triticina* are binucleate.

It may well be that one should extend Lindfors' recommendation to cover this case and consider it equivalent to the binucleate condition because it begins and ends binucleate. Even so, it remains unexplained why the number of nuclei here should be predominantly and almost uniformly *three* through all but the youngest vegetative development.

The production of regularly uninucleate haustoria from trinucleate haustorium mother cells is not far out of line with other rusts, but thus far the writer has found no exact parallel in the literature.

Sappin-Trouffy (*32*), in extensive studies of representative genera throughout the Uredineae, found regularly uninucleate haustoria produced by mycelium composed of uninucleate cells, and either uninucleate or binucleate haustoria (or both) produced by mycelium composed of binucleate cells.

Holden and Harper, in 1903 (*19*), found that the haustoria of *Coleosporium sonchi-arvensis* were regularly binucleate, like the mycelium which bore them.

Ward, in 1903 (*34*), studying the mycelium of *Uredo dispersa*, drew one to six nuclei in vegetative cells, two or three nuclei in haustorium mother cells, and one or two (usually one) in haustoria.

Blackman, in 1904 (*4*), working on *Phragmidium violaceum* found paired nuclei in haustoria of binucleate mycelium. Blackman and

Fraser, in 1906 (*5*), found two nuclei in haustoria in the binucleate mycelium near the teliospores of *Uromyces ficariae*.

Pole-Evans, in 1907 (*15*), described the uredo mycelium of *Puccinia phleipratensis* as having two or more nuclei in the haustorium mother cell and two in the haustorium. The multinucleate cells of *P. glumarum* produce haustoria with as many as five nuclei. In the uredinial mycelium of *P. dispersa* a uninucleate haustorium is figured.

Dodge, 1918 (*11*), speaking of the telial mycelium of *Gymnosporangium macropus*, said: "The haustoria take a very delicate stain, showing the single nucleus in each, or two nuclei when two are present."

Thurston, in 1923 (*33*), studying *Gymnosporangium bermudianum*, found uninucleate haustoria from binucleate mycelium.

The present writer, in 1923 (*3*), found one nucleus in the haustoria of *Puccinia graminis tritici*.

This summary is not complete, but as far as it goes it would indicate that the haustoria of different rusts have from one to five nuclei; that the number is sometimes variable, even on the same mycelium; and that the number of nuclei in the haustoria is either the same as that of the mycelium bearing them, or somewhat less. The situation in *Puccinia triticina* physiologic form 11 is in line with this, in that the number in the haustoria is reduced but appears to be more definite and limited than in the similar cases cited.

The significance of the joining of the rust sporelings at the stoma is not known. Not all of the literature has been covered, but no case like it has been noted. The frequency of its occurrence naturally depends, for one thing, on the number of appressoria present. An abundance of spores was used in the inoculation of this material, but some stomata were free, many carried one sporeling, and comparatively few carried more. Even when appressorium are grouped at a stoma they may remain separate and enter and develop independently of each other. Of all the rust sporelings studied in this material, about 4 per cent showed fusion.

Not enough is known of the details of the fusions at the stoma and when spore bearing is imminent, to determine whether they have sexual significance or are merely anastomoses of a vegetative nature. Pairing of nuclei or nuclear fusion has not been proved.

Even a brief survey of rust cytology suffices to impress one with the plasticity of the nuclear history as shown in rusts with altered and abbreviated life cycles. Blackman (*4*), Dittschlag (*10*), Fromme (*16*), Lindfors (*24*), and others thought that even the regular process of fusion at the base of the aecium was a substitute for an earlier form of reproduction in which the pycniospores functioned as male cells, an opinion that is opposed by Christman (*7*). In rusts without an aecium a very similar fusion occurs in the first uredinial generation (*8*) and in certain of the micro- and lepto-forms fusion occurs at the base of the telium (*24*). Moreover, the fusion is not necessarily between the basal cells which give rise directly to spores, as it may occur several cell generations earlier in the vegetative mycelium (*24*). Morphological evidence of the plasticity of the rusts is given by Dietel (*9*), who traces the derivation of reduced types from the corresponding heteroecious or autoecious forms. The teliosori of the reduced species occur in small or large groups like the *aecia* of the corresponding heteroecious type and bring about the same deformation of host tissues.

In an Endophyllum, Hoffmann (*18*) found an aecium of normal appearance producing aeciospores which germinated like teliospores by producing promycelia the sporidia of which lead directly to more aecia.

Recent work by Kunkel (*21*, *22*, *23*) and Dodge (*12*, *13*) on the orange rusts of Rubus shows that side by side with the regular long-cycle rust (O, I, III) there occurs a short-cycle form in which the aeciospores germinate by forming promycelia, thus returning directly to the uninucleate condition and eliminating the telium. Moreover, according to Dodge (*13*, *p. 494*), there is some evidence of a "third or intermediate type which is maturing aecidia of two kinds," and which "may represent a strain of Gymnoconia which is particularly unstable and from which a short-cycled rust is now arising and which will have distinct morphological characters of its own."

These instances show the plasticity of the rusts in being able to shift the processes of fusion and reduction from one part of the life cycle to another, and the persistence of the processes themselves under varied conditions and alterations of the life cycle. Isolated instances occur where both fusion and reduction appear to be lost, as in the Endophyllum whose mycelium and spores are all uninucleate (*26*) and the uninucleate microform *Uromyces Rudbeckiae* (*30*), but these are exceptional.

Nor is such plasticity limited to rusts. In *Aspidium falcatum*, an apogamous fern (*1*), the sporophyte arises by a vegetative outgrowth from the prothallium without a fusion of nuclei. The "haploid sporophyte" so formed is normal in all morphological respects. In the sporangium 16 spore mother cells form, as in other ferns. These fuse by pairs to form 8, which then undergo reduction divisions, forming a maximum of 32 spores. Here the sexual process is shifted to the opposite end of the life cycle.

It would be interesting to know whether the aberrations in nuclear behavior observed in *Puccinia triticina* physiologic form 11 are to be regarded as an expression of the need of, and an ill-defined attempt at compensation for, the excised part of the life cycle. Also whether ultimately a revised nuclear history is to be expected, such as has been achieved in other rusts and by means of which *P. triticina* would be rendered independent of any aecial host.

Rusts represent the extreme of obligate parasitism. The success of the rust varies directly with the vigor of the host. The food of the fungus is obtained by means of haustoria which invade the *living* host cells. When the death of host tissues occurs in the infection the rust is weakened or even killed, for it can not subsist on decaying tissues. Susceptibility thus depends, for one thing, upon the ability of parasitized tissue to live and continue functioning more or less normally in company with the fungus.

Infections of *Puccinia triticina* physiologic form 11 on Little Club present an almost perfect picture of susceptibility, and this varies little with the age of the host. The fungus produces the minimum of disturbance in the host tissues. Even the first cell attacked survives, although on other hosts slightly less susceptible the first cell invariably succumbs. The walls of host tissues are not disintegrated, and the tissues are not plasmolyzed. The invaded tissues have somewhat heightened turgor, and their nuclei are slightly expanded. Under conditions favorable for the growth of the host, starch formation

proceeds at such a pace that the plastids have a normal or slightly greater than normal content of starch, in spite of the inroads of the fungus.

The presence of normal starch content in infections when host and rust are growing vigorously and apparently well adjusted to each other perhaps was to be expected. The presence of excess starch in infections on weakened leaves was a surprise. The leaves of this set received more sunshine, but this can hardly explain the difference, for uninfected tissues in 1924 and 1925 had similar starch content. (Table 2.) A possible explanation is that the fungus stimulates the photosynthetic activities of the host. Under good conditions, the excess starch is carried away. Under unfavorable conditions, some phase of the machinery of translocation breaks down and the starch accumulates. This excess starch apparently is utilized by the fungus; at least it is absent where the fungus is densest in old infections and is to be found then only in the marginal regions of the infection.

Rand's observations on pecan rosette (*31, p. 25, 26*) afford an interesting parallel in a widely different type of disease. Healthy leaves contained a moderate amount of starch at sundown and were starch-free the next morning. Mottled leaves showed "profound derangements * * * in starch assimilation and translocation." He says: "Mottled leaves of all sizes collected at night showed the green portions gorged with starch * * * swollen as if almost bursting with their accumulation of starch * * * Mottled leaves collected before sunrise the following morning appeared the same as those collected at night." It would be difficult to say what there is common to the two cases, save a possible breakdown in translocation.

SUMMARY

Puccinia triticina Erikss. is a heteroecious rust with uredinia and telia on wheat, and with aecia on exotic species of Thalictrum. In America the rust exists mainly if not exclusively as a continuous series of uredinial generations.

Puccinia triticina physiologic form 11 enters Little Club wheat freely. Forty hours after inoculation only 37 out of 204 fungi (about 18 per cent) still remained outside. Two appressoria at the same stoma may become united into one, and, when the two enter separately, the substomatal vesicles may fuse. About 4 per cent of the fungi studied showed this fusion.

In general, uredinial generations of rusts are made up of binucleate cells and are considered sporophytic. In *Puccinia triticina* physiologic form 11 the urediniospore has 2 nuclei, the appressorium usually 4, the substomatal vesicle commonly 8, the first hypha, after forming a haustorium, has 6 nuclei, one or two succeeding hyphae 4 or 5, and in all the later vegetative cells the number 3 predominates. Haustorium mother cells have 3 nuclei and haustoria have 1.

At the onset of reproduction the subepidermal mycelium concerned with spore production becomes binucleate. Spores, stalk cells, and the cells immediately below them regularly contain two nuclei.

Two cases of the fusion of hyphae were noted in the region where spore formation is imminent.

It is suggested that the irregularities in the nuclear history may have some connection with the prolonged growth of the rust in the absence of the aecial host.

Little Club wheat is fully susceptible to *Puccinia triticina* physiologic form 11. On Little Club wheat the fungus attains its maximum development, the haustoria expand fully, and spores are produced in abundance. The host tissues live, their cell walls (except in the guard cells at the stoma of entry) are not damaged, and the cell contents show a minimum of disturbance or impoverishment. The cells in the infection are more turgid, and their nuclei expand somewhat and move over to the haustoria. At the period of greatest activity of the fungus the plastids show little reduction in size. Under unfavorable conditions they may be temporarily gorged with starch. Not until extreme old age of the infections, do more than 1 or 2 per cent of the host cells die. In the more massive fungous growth on older host plants a few subepidermal mesophyll cells are crushed by the fungus. On the whole, the picture is one of excellent congeniality.

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EFFECT OF TEMPERATURE AND MOISTURE ON NEMATODE ROOT KNOT [1]

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INTRODUCTION

Root knot, one of the most destructive of plant diseases, seems to be limited in its distribution largely by climatic conditions. Comparatively little work has been done on the fundamental problems related to the influence of temperature and moisture on the disease and its causal organism. This paper deals with experimental work on some of these problems; and the writer believes that some of the results of this work may be of practical economic importance to commercial greenhouse men and truck growers in the curtailment of losses due to root knot in celery, lettuce, and other low-temperature-loving plants.

THE DISEASE

Root knot attacks a wide range of host plants representing several hundred distinct species. These include practically all the common vegetables, many field crops, and even some fruit trees. The principal symptoms are enlargements or galls on the roots, varying in size from a pinhead in mild cases to a walnut where infestation is severe and complicated by the development of new generations of the parasite within the host near original points of infection. The causal organism is the nematode or eelworm *Heterodera radicicola* (Greef) Müller, a minute worm-shaped animal scarcely a millimeter in length at maturity. The galls formed as the result of stimulation of the host by the presence of the organism or some product excreted by it interfere greatly with the normal functioning of the roots; and the nematodes, which are ordinarily present in enormous numbers, feed entirely upon the host plant juices. These two effects together may seriously influence the metabolism of the host plant. In severe cases the plant is very much dwarfed or even entirely killed.

RELATION OF CLIMATE TO DISTRIBUTION OF ROOT KNOT

The nematodes are readily carried in soil clinging to bulbs or roots of plants, as well as within such plant tissues. They have been distributed widely throughout the country, north as well as south. Except in greenhouses, however, the disease is limited in its serious agricultural importance in the United States to the Southern and to some extent to the border States. It is at its worst in the extreme South. It is recognized as a factor in agriculture, however, in

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Virginia, and reports are more and more frequent of its occurrence in Maryland, Delaware, and New Jersey. Southern Ohio, Indiana, and Illinois are finding it extensively in truck gardens and nurseries. Most of the Mississippi Valley States from Iowa northward do not have root knot to combat. Only occasionally is it to be found in small areas which are unusually favorable. In the northern tier of States in this section it is unknown outside the greenhouses. West of the Rocky Mountains, in Idaho, Oregon, and Washington, the disease is occasionally found.

The limiting factor on distribution seems therefore to be principally that of climate. Temperature may have its effect in two ways: (1) In its limitations on the actual life of the organism causing the disease, and (2) in its effect on the number of generations that may be produced even if the organism can remain alive throughout the year.

The earliest American literature on the subject recognized, in a general way, the limitations placed by severe winters on the distribution of root knot. Neal (*6*) stated that "the question of temperature is no doubt one of great importance in determining the boundaries of this disease, perhaps more so than food plants or soils." He stated that soil that is annually frozen from 6 to 10 inches is rendered practically free of the worms, except where they occur on perennial roots. Below 50° F. the worms were inactive, "paralyzed by cold." He cited the occurrence of the disease only in the South Atlantic and Gulf Coast States. Atkinson (*1*) referred to accounts of the disease in Germany, Java, Brazil, and in Scotland. He emphasized this latter occurrence as evidence against Neal's limitation of 50° F. winter temperature, and prophesied that if the pest once became established it would probably thrive as far north as the January isotherm of 35° F., which crosses Virginia and Tennessee. Marcinowski (*5*) included in the list of countries where the disease occurred (besides the central and southern European countries) Brazil, Chile, South Africa, Egypt, Madagascar, Sumatra, and Java, mostly countries with warm climates. Stone and Smith (*7*) considered freezing fatal to the root-knot nematode. Bessey (*2*), on the other hand, found root knot in northern Indiana, Michigan, and Nebraska, and considered that the nematode was able to maintain itself in regions where the winter's cold was very intense.

Aside from the question of freezing out in the winter, however, in the Southern States the long warm summers combined with favorable soil and moisture conditions bring about the development of several generations in a single growing season, thus greatly increasing the nematode population of the soil. Farther north, the shorter summer season shortens the period during which the nematodes can be active and therefore limits their increase.

Moisture has its effect principally in that it limits the growth of suitable host plants. Root knot occurs on many weeds in uncultivated lands in Georgia, Florida, and South Carolina, where there is continuous summer rainfall. In the Southwest, on the other hand, it is never found except in irrigated fields, the limiting factor being moisture and host availability, and not temperature.

Neal (*6*) stated that an excess of moisture was conducive to the disease, and that drainage was a remedy under such conditions. He also stated that moisture was essential to the vigorous growth

of the nematode, although it withstood an enormous amount of drying. Stone and Smith (*7*) agreed in general as to the need of moisture, and even said that nematodes do not seem to suffer from an excess of it, but stated that neither the nematodes nor their eggs can stand desiccation. In their experiments, soil in jars was allowed to dry out for a year, and at the end of that time the nematodes were not alive. Bessey (*2*), Frank (*3, pl. III*), and others conducted experiments which indicated that the larvae of the nematode were readily killed by drying. Excess of moisture, according to Bessey, does not seem to inhibit the organisms, unless the land is completely flooded and the organisms are loose in the soil and not protected by the roots of perennial plants. The flooding must be for at least 25 days to be efficacious in destroying the nematodes.

Considerable observational information is given in the papers cited, some of it more or less contradictory. Very little actual experimental work has been described, and practically no clear-cut conclusions reached. The subject of the influence of specific moisture conditions and temperatures on the development of the disease is left practically untouched. The present paper gives the results of definite experimental work which has cleared up conclusively some of the doubtful questions with regard to the relation of moisture and temperature to the development of root knot.

EFFECTS OF SOIL MOISTURE AND SOIL TEMPERATURE ON DEVELOPMENT OF ROOT KNOT

EXPERIMENTAL METHODS

Specific experiments for the purpose of determining the effects of soil moisture and soil temperature on the development of root knot were conducted at Madison, Wis., and Washington, D. C., in 1921, 1922, and 1923.

The Soil

Throughout the history of observations on root knot under field conditions, it has been known that the disease is usually at its worst in sandy soils. Consequently, sandy soil was determined upon at the outset for this experimental work. Usually virgin soil, composed largely of peat to which some manure was added or rich compost ordinarily used in the greenhouse, was made the basis, and to this about half the same volume of sand was added. This gave a rich sandy soil very favorable to the development of the root-knot nematode. Inoculation of the soil was accomplished by adding quantities of knotted roots of plants, found upon close examination to contain an abundance of eggs and larvae of *Heterodera radicicola*. Figure 1 shows the lower part of a French cocklebur plant (*Urena lobata* L., a weed common in Florida, highly susceptible to root knot), which was used for a large number of the inoculations.

Moisture Content

Standards for the determination and maintenance of soil moisture content were determined (1) by sampling in triplicate the thoroughly mixed soil and by oven-drying at 100° to 105° C., determining the dry weight, and (2) at the same time the percentage of moisture on the dry-weight basis necessary for complete saturation was determined, also in triplicate, and the average determined. The desired

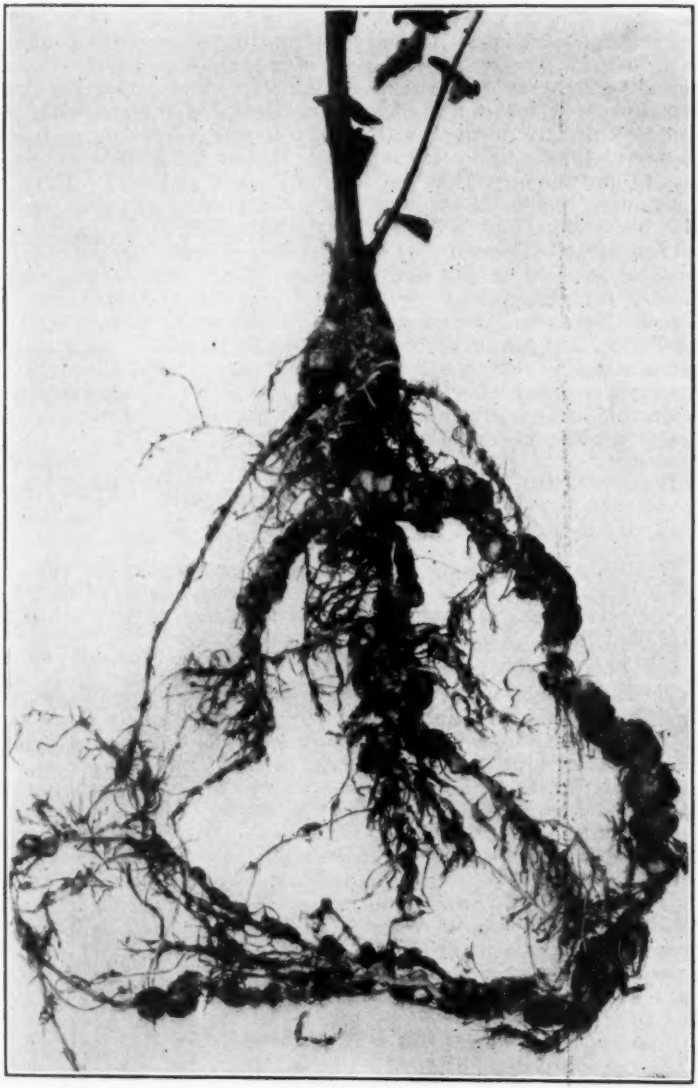

FIG. 1.—Root of French cocklebur (*Urena lobata* L.), badly infested with root knot. Material such as this was used as source of inoculum for much of the soil-moisture and soil-temperature work. The larger discolored knots contain myriads of eggs and larvae of the root-knot nematode

moisture in percentage of saturation moisture was then easily determined. For the early part of the soil-temperature work, a medium amount of moisture, usually 50 or 60 per cent, was maintained, this having been known from general observations to be favorable both to plant growth and development of the nematodes.

Temperature Control

Soil-temperature control was accomplished by the use of the "Wisconsin soil-temperature tanks," a history of the development of which has recently been written by Jones (*4*). A new set of six tanks was built in the fall of 1921 for this purpose. The latest improvements were incorporated in them. During the course of the experiments, records were taken of the temperatures twice a day, morning and evening, and any irregularities were corrected at those times.

The readings were taken at a depth of about 5 inches in the water tanks. Variations of from 1 to 1½ degrees were noted in different parts of a tank, which contributed, of course, to the inaccuracies of recorded temperatures. No device was used in any of these experiments to circulate the water in the tanks.

In general, temperatures within 1° C. in either direction were maintained regularly. Any great discrepancy was due to either the failure of the set of batteries which controlled the relays, thus allowing the temperatures to rise above the desired point, or to the burning out of a fuse controlling the heaters, which permitted the temperatures to fall. When anything of the kind happened, the correction was usually made within a few hours of the break, so that for practically the entire length of the experiments the temperatures averaged within the desired range for each tank.

Duration of Experiments

Experiments were continued as a rule for a 30-day period. Under the most favorable conditions this permitted the development of only one entire life cycle, from egg or larva in the soil to infection and growth to the adult stage within the host plant. Consequently one condition was completely comparable with another in the matter of primary infections. No secondary infections, from the young of matured individuals developing under the most favorable conditions, entered into the results, except in those experiments in which the duration of exposure was definitely longer than 30 days.

Manner of Recording Results

The results of a series of differences in one controlled environmental factor were to a certain extent evident in the tops of the plants. Figures 2 and 3 show these differences strikingly in the case of moisture control, the one in effect on the growth of the plants, and the other in relative growth under specific conditions as compared with the controls under the same conditions. Figure 4 shows the differences where the condition controlled is the temperature.

However, since considerable irregularities exist between different individuals under the same conditions, this is obviously not a sufficiently reliable basis for drawing conclusions on the effect of

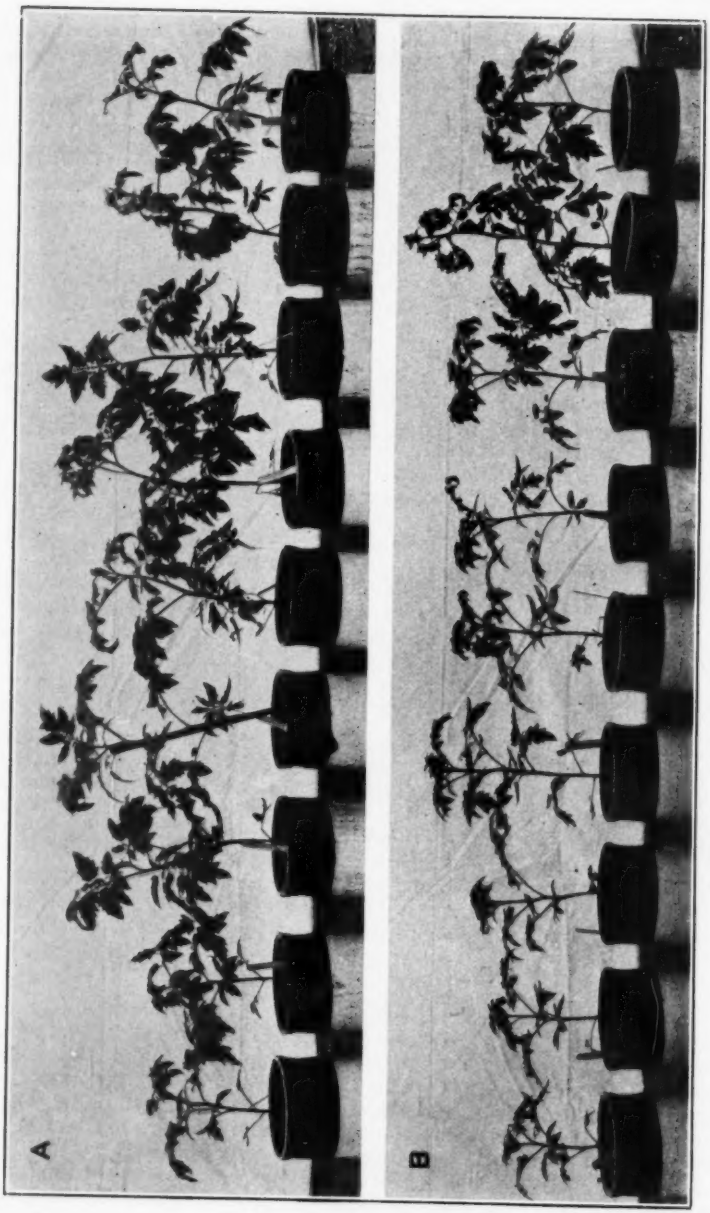

FIG. 2.—Tomato plants at the close of the controlled soil-moisture experiment 2
(For explanatory legend see page 229)

environmental factors on root knot. The most accurate way would be to determine the actual number of larvae that penetrated the roots under each condition. While this was perhaps possible, it was impracticable. The numbers under some conditions were so large and the difficulty of getting the organisms out too great to permit of it being done. It was decided, therefore, to make comparisons on the basis of number of galls per pot. Under some conditions infections were comparatively few, and individual galls could be counted very readily. This count was accomplished, as a rule, by placing the entire roots, which had been carefully removed from the soil at the close of an experiment and washed free from adhering dirt, into a large glass dish of water, over a black-surfaced table. Then with a pair of tweezers, observations could be made on each root separately, and counts made with an automatic counter. Under somewhat more severe conditions, galls were so numerous as to produce a beaded effect on many of the roots. Here again counts could be made very readily. Under still more severe conditions, however, galls coalesced, and individual counts could not readily be made. It would certainly not be legitimate to count a very small gall as the equal in value of a very large one, one possibly 20 times the size and containing many nematodes. It was arbitrarily decided in such cases to count each millimeter of the elongated swelling as one gall. As a rule, this appeared to give results roughly comparable with those of other conditions. In several cases even this means did not give results that reliably indicated the actual effect of root knot on the host plant. Some plants that were obviously severely injured gave a smaller total of knots than some that were less severely injured. In some cases, therefore, the results are given also in number of galls per unit of length of root. The total linear measurement of roots was taken, only the larger ones being measured. Very fine roots less than 2 centimeters in length were ignored. This method seemed to indicate accurately and reliably the relative injury done under the different conditions.

EFFECTS OF DIFFERENCES IN SOIL MOISTURE ON DEVELOPMENT OF ROOT KNOT

Aside from effects of flooding and of drying on the life of the root-knot nematode, only general observations have heretofore been made on the effect of degree of wetness of the soil on the development of root knot. These observations have indicated a relatively wide range within which the nematode is active in attacking the host plant. It was deemed desirable to make tests to determine definitely the range of activity of the organism in this respect.

EXPERIMENT 1

A preliminary experiment was conducted at Madison, Wis., in March, 1922, using tomatoes and soy beans. The usual sandy loam

EXPLANATORY LEGEND FOR FIGURE 2

A.—The control series, grown in sterile soil, moisture contents from left to right being 28 (approximate), 30, 40, 50, 60, 70, 80, 90, and 100 per cent of saturation. Very little growth occurred during the period of the experiment (one month) in the first two. Good vigorous growth occurred in the rest of the series, not much difference in growth being evident in the tops of the plants.

B.—The inoculation series arranged in the same order as the control series, and with exactly the same reduction. Note the greatly decreased vigor of the plants in the middle of the series.

FIG. 3.—Tomato plants grown for a period of two months under controlled soil-moisture conditions

A.—Forty per cent of the moisture-holding capacity, two inoculated and one control.
B.—Sixty per cent of moisture-holding capacity, showing even greater contrast between the inoculated plants and the control. The plants are somewhat larger at 60 than at 40 per cent.

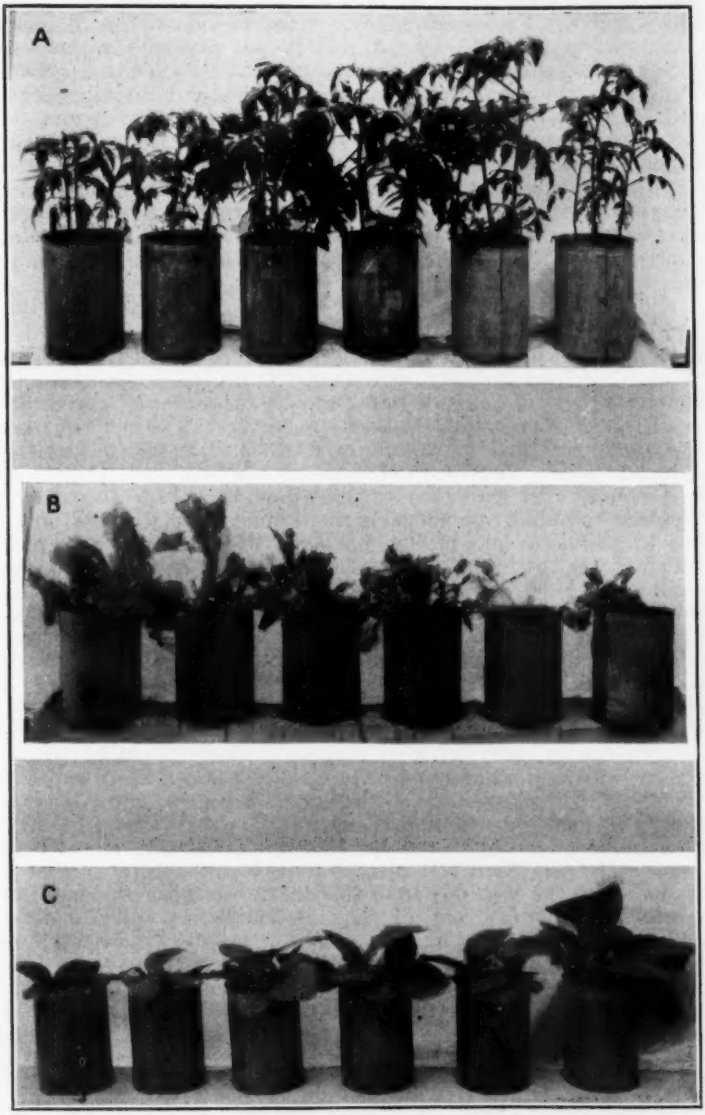

FIG. 4.—The effect of temperature on the growth of various plants

A.—Tomatoes grown in experiment 3, the temperatures, from right to left, being 15°, 20°, 25°, 30°, 35°, and 40° C.

B.—Lettuce from the same series, showing strikingly the reversal in effect of temperature from that shown in *A*. Soil fungi were partly responsible for this, however.

C.—Tobacco grown in a cool house, at soil temperatures of 10°, 13°, 16°, 19°, 22°, and 25° C. respectively.

(greenhouse compost plus sand) was used, lightly inoculated with nematode-infested dasheen material from Florida. The moistures maintained were 40, 50, 60, 70, and 80 per cent of the moisture-holding capacity of the soil. The lowest contained sufficient moisture to maintain plant growth to good advantage, and the highest was only slightly too wet. Water lost by transpiration and evaporation was replaced twice daily, morning and evening. One series was inoculated soil, and another series control.

The results were irregular and indicated the necessity of repeating the experiment under better conditions, particularly with a longer series of moistures, and with a larger number of inoculated plants under each condition. The results of experiment 1 are not recorded here.

EXPERIMENT 2

An experiment incorporating these desiderata was started at Washington, D. C., about November 1, 1922. Glazed crockery jars of about 2 gallons capacity were used. A sandy loam consisting of about two parts of compost and one part of sand was sterilized in the autoclave, then set out to aerate for several days in a large box. After a thorough mixing, samples were taken in triplicate for determining moisture content and moisture-holding capacity. Then nine jars were filled with even weights of soil, these to be the controls. Nematode material was added to the remainder of the soil, in the form of shredded and broken up badly diseased roots of French cocklebur (fig. 1) from Brooksville, Fla. The material contained an abundance of living nematodes, mostly in the larval stage. Twenty-seven jars were filled with this material to the same weights as the controls. This allowed for three inoculations and one control for each of the following percentages of moisture-holding capacity: 10, 20, 30, 40, 50, 60, 70, 80, and 90.

Vigorous young tomato plants were set out at once. They were permitted to stand under equal conditions for two days, while the moisture content was being determined. The content at time of planting was approximately 40 per cent of water-holding capacity. At the end of the two days the higher moistures were made up by adding the requisite amounts of water. It was 14 days, however, before sufficient moisture was lost from the lower pots to reach the 30 per cent mark, and the 20 and 10 per cent points were never attained. On the 15th day from the start, the 10 per cent jars were made over to 100 per cent, or complete saturation. The moisture content of the 20 per cent jars at this time was somewhat lower than that in the 30 per cent jars, and the 20 per cent jars were maintained throughout the experiment without the addition of any water.

Several extra jars were planted at the time the experiment was started and maintained at 40 and 60 per cent of saturation. These were utilized for the determination of accrued weights brought about by plant growth from week to week, these weights being added to the various jars to make up for the substance of the plants themselves. The accrued weights were estimated for pots other than those containing the 40 and 60 per cent moistures. The total accrued weight by the third week did not exceed 20 grams, so estimates were well within the allowable divergences. (The aim was

to keep weights within the range of 5 per cent below the percentages fixed upon. Thus, when the 60 per cent jars first lost enough moisture (76 grams) during the course of 24 hours to make it 55 per cent at its lowest point, water was added to bring it up to the desired weight twice daily thereafter; and similarly with the rest of the jars.) The jars were arranged on the greenhouse bench four deep from front to back, and were rotated in their relative positions with reference to light by shifting the front rows to the back and bringing the rest forward once each day. Thus all had approximately equal environmental conditions aside from moisture. Greenhouse temperatures ranging from 15° C. at night to 20° C. in the daytime were maintained.

On December 7, just 30 days after beginning it, the experiment was brought to a close. The effect of differences in moisture content was striking, especially in the control series. Very little growth occurred at 30 per cent of moisture capacity or below. From these points growth gradually increased with each rise, even up to 90 per cent, except for some variations due to individual differences at the intermediate points. Height is not a good criterion of "best growth," since at the highest moistures the growth is weak and succulent, and contains less ash than at the medium conditions. This fact is considered in drawing conclusions. Figure 2 shows the control series ranging from 25 or 26 per cent moisture to 100 per cent saturation. Differences between inoculated and controls at the same moisture content were also striking, especially in the middle of the series. This is seen by comparing Figure 2, A (the control series) with Figure 2, B (the inoculation series). Growth was very much reduced at from 50 to 80 per cent in the inoculated jars, while it was at its best in the controls. These differences are seen still more strikingly by comparing all three of the inoculated plants with the controls, as in Figure 3, A and B, showing 40 and 60 per cent jars, respectively.

As stated under "Methods," a comparison of the root systems entered into the ultimate results. Marked differences in type of root growth were noted. At the lower moistures roots were irregular, bent, gnarled, and in intimate contact with the soil particles, from which several washings would not free them. Root-hair development was prominent. At the higher moistures roots were smooth, straight, and clean, and root hairs were not evident (fig. 5, A). Differences in extent of root growth, as influenced by amount of soil moisture, were also prominent. In dry soil the roots extended to the bottom of the cans and became matted there, while in the moist soil they extended scarcely to the bottom. Fairly exact comparisons were made by measuring the total lengths of all the separate roots, except the very fine feeding thread roots and the short branches that were just developing. These measurements are recorded in Table 1. Typical roots from inoculated plants, as compared with controls, are shown in Figure 5, A, B, and C. Root-knot results are given in Table 1 in total number of galls per plant, and average number per plant for each condition. In addition, average numbers of galls per decimeter of length of root are tabulated. The graphs in Figure 6 bring out the same results more sharply. The table also shows the heights of plants in the entire series, in centimeters.

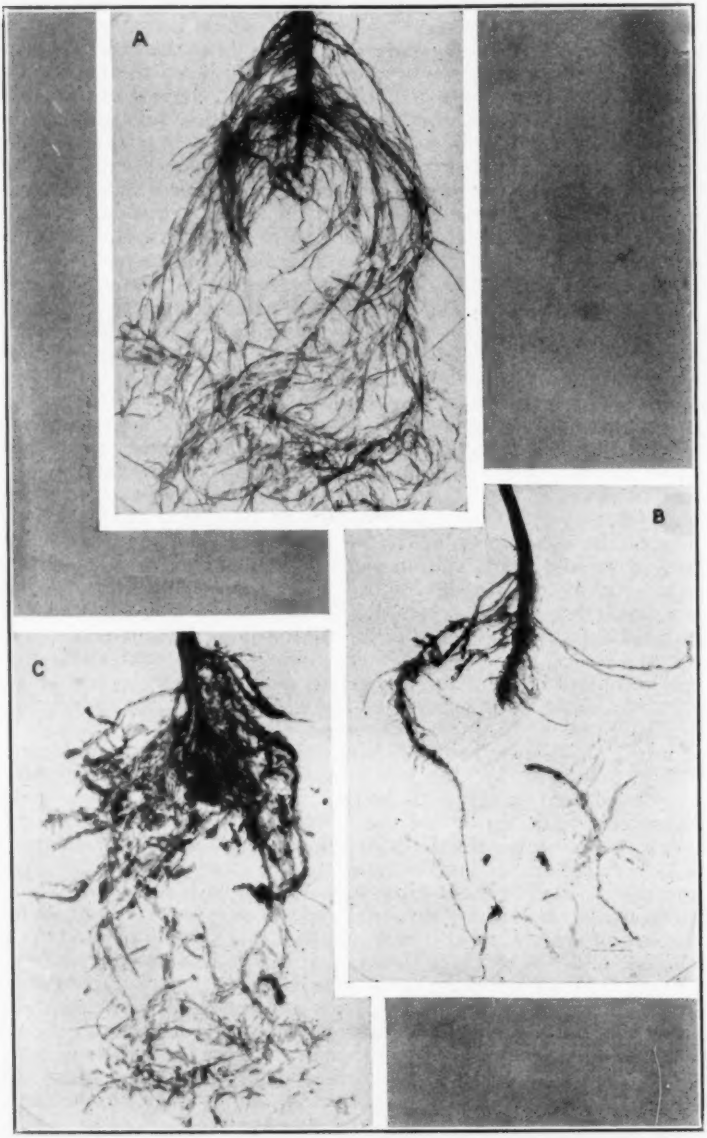

FIG. 5.—Tomato roots after two months in soil, at 60 per cent of the moisture-holding capacity

A.—Root of control plant, clean and healthy.
B.—Root of one of the inoculated plants, showing a large proportion of the root killed, owing to severity of root knot.
C.—Root of the other inoculated plant, showing severe infestation, but the greater part is still alive. A comparison of B and C shows why a count of the number of knots on the roots is not an accurate criterion of the severity of infection.

TABLE 1.—*Effect of soil moisture on root-knot development in tomatoes*

Moisture (per cent of saturation)	Control		Series A				Series B			
	Height of plants	Length of roots	Height of plants	Length of roots	Number of knots	Number of knots per dm.	Height of plants	Length of roots	Number of knots	Num ber of knots per dm.
	Cm.	*Cm.*	*Cm.*	*Cm.*			*Cm.*	*Cm.*		
28 to 30 [1]	27	1,084	22	405	195	4.81	30	1,043	818	7.84
30 to 35 [2]	30	1,098	20	309	787	25.46	30	565	416	7.36
40	41		32	706	1,257	17.80	27	520	959	18.44
50	50	805	28	343	705	20.55	35	840	1,463	17.42
60	51		35	889	1,786	20.08	35	1,040	1,859	17.88
70	45	809	32	598	1,864	31.17	32	478	823	17.22
80	37	628	36	781	2,136	27.34	33	227	152	6.70
90	37	492	45	1,046	1,366	13.05	44	666	1,181	17.73
100 [3]	34	368	30	361	641	17.75	33	375	346	9.23

Moisture (per cent of) saturation	Series C				Averages of series A, B, and C			
	Height of plants	Length of roots	Number of knots	Number of knots per dm.	Height of plants	Length of roots	Number of knots	Average number of knots per dm.
	Cm.	*Cm.*			*Cm.*	*Cm.*		
28 to 30 [1]	28	611	1,068	17.48	27	686±102	694±124	10.04±2.10
30 to 35 [2]	31	543	643	11.84	27	472±45	615±51	14.89±3.00
40	33	750	1,617	21.56	31	659±39	1,278±28	19.27± .64
50	41	883	1,614	18.28	35	689±95	1,261±134	18.75± .51
60	39	663	1,661	25.05	36	864±60	1,769±27	21.00±1.23
70	37	410	593	14.46	34	495±30	1,093±186	20.95±2.85
80	35	551	1,124	20.40	35	520±88	1,137±273	18.15±3.34
90	50	868	1,394	16.06	46	860±60	1,314±31	15.61± .84
100 [3]	40	398	243	6.11	34	378±6	410±57	11.03±1.92

[1] This did not get down to 30 until the experiment was half completed. No water was added during the experiment.

[2] Not down to 30 until experiment was half completed.

[3] Changed from approximately 30 per cent half way through the experiment. No doubt much of the infection occurred before the change was made.

Table 1 shows, in the controls, a definite decrease in length of roots, as amount of moisture is increased. This regularity does not hold with the inoculated plants, where another factor, root knot, enters in. Figure 2, and the data for top growth in Table 1, show that saturation below 40 per cent and above 70 per cent are not favorable to the best growth of the plants. Within the range of best growth, and even above it up to 90 per cent, the root knot is at its worst. This is shown by both curves in Figure 6. At 70 and 80 per cent the average number of knots dropped off, but the number per unit of length of roots is highest of all, showing apparently greater

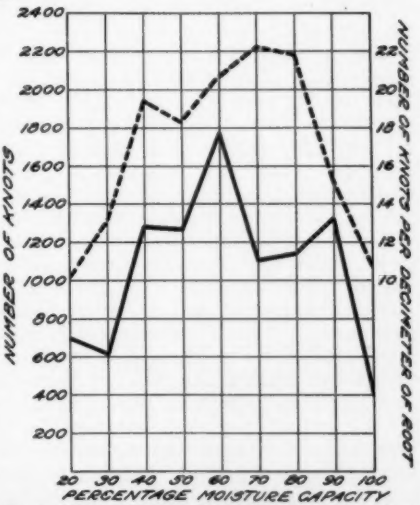

FIG. 6.—Effect of moisture on development of root knot in tomatoes. (The unbroken line represents number of knots; the dotted line, number of knots per decimeter of root.)

injury by the root knot there than at the other moistures. The number of individuals under each condition, in this experiment, was so small and the probable errors so large that not much significance can be attached to the differences shown between adjacent moisture contents. The general conclusion that within the range of best growth of the host plant the nematode is most active is obvious. This will probably hold true for most of the ordinary susceptible plants that are grown under root-knot conditions. The soil moisture, under growing conditions, fluctuates considerably, of course, but it is undoubtedly between 40 and 70 per cent of saturation the greater part of the time.

EFFECTS OF SOIL TEMPERATURE ON ROOT-KNOT DEVELOPMENT

Preliminary experiments on effects of soil temperature on root knot were conducted in the greenhouses of the department of plant pathology, University of Wisconsin, Madison, Wis., in the fall of 1921. These were followed by more conclusive experiments at Madison and also at Washington, D. C., during the early spring of 1922 and in the winter of 1922–23.

EXPERIMENT 3

The basis of the soil used in this experiment was greenhouse bench soil which had borne a crop of badly root-knotted cucumber during the summer. To this was added about half its volume of sand to improve its texture and make it more favorable for nematodes. Additional inoculum was added in the form of root-knot-infested cowpea roots and dasheens from Florida. Control soil was made up from similar bench soil that had not borne root-knot affected crops. Moisture was maintained according to the usual procedure at 50 per cent of the moisture-holding capacity of the soil, which degree of moisture was chosen because of its obvious suitability for growth of the plants. The plants used were lettuce and tomatoes, three inoculation cans and one control of each per tank. Three healthy plants, from 2-inch pots, were set out in each can. Temperatures maintained were 15°, 20°, 25°, 30°, 35°, and 40° C. The daily records show no very great fluctuations from the desired temperatures during the period of the experiment, November 18 to December 18, 1922. On December 4 the tanks dropped from 2° to 5° (the higher temperatures dropping the most), and three days before the close of the experiment the highest tank went up 3°. Aside from these fluctuations, which lasted at most only four or five hours, variations from the desired temperatures were only 1½° or 2° and were corrected twice daily.

This experiment, with 5° intervals, was conducted in part for the purpose of determining cardinal temperatures for the growth of the plants. Results in vigor of growth in the case of the tomatoes are shown in Figure 4, A. Poorest growth resulted at the lower temperatures. Top growth increased with the higher temperatures, up to 35° C. At 40° the plant remained alive and even grew slightly, but very little root growth was made. In the matter of root growth, results were somewhat contrary to what might have been expected from the appearance of the tops. Roots were vigorous at the lowest

temperature (15°), occasional strands extending to the bottoms of the 9-inch-depth cans. At 20° root growth was somewhat more extensive, while at 25° and 30° it was very vigorous, with heavy roots matted in the bottoms of the cans. At 35°, however, root growth was much reduced, even though top growth was greatest at this temperature. At 40° there was practically no root growth outside the ball of the soil in which the plant was introduced into the cans.

Root growth in inoculation cans was approximately the same as in the controls.

Results in root-knot development were recorded in number of knots per can. The figures, including the averages, are given in Table 2. The average results are also shown in Figure 7.

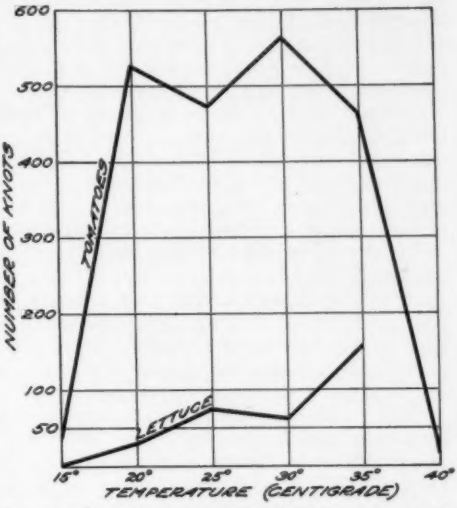

FIG. 7.—Effect of temperature on development of root knot in tomatoes and lettuce

TABLE 2.—*Effect of soil temperature on development of root knot in tomatoes and lettuce*

Tank No.	Temperature, degrees C.	Tomatoes			Lettuce		
		Number of knots			Number of knots		
		Plants in first can	Plants in second can	Average	Plants in first can	Plants in second can	Average
1	15	20	54	37± 8	0	4	2± .95
2	20	617	431	524±44	25	33	29± 1.9
3	25	343	607	475±63	105	41	73±15.3
4	30	548	573	560± 3		59	59
5	35	471	460	466± 3	152		152
6	40	10	21	16± 2			

It is evident that at 15° C. the amount of root knot is negligible. It is very abundant at 20°, indicating a rather sharp point below which infectio does not take place. From 20° up infection is abundant up to the upper limit of heat endurance by the host plant. The few knots that are recorded for 40° were near the surface, where the temperature was probably somewhat lower than in the interior of the cans.

In lettuce the effects of temperature on growth of the plants, as shown by the controls, was almost the reverse of what it was with tomatoes. By far the best growth resulted at the lowest temperature, and as temperatures increased growth and vigor of

the plants decreased. Another factor besides temperature entered into the case of lettuce. As already stated, the soil was not sterilized. As a result of the presence of the lettuce drop fungus, *Sclerotinia libertiana*, one of the root-knot-inoculated plants each at 30° and 35° C., and one at 30° in a control, were killed, and several others weakened. Figure 4, B, shows a series of lettuce plants in control cans, illustrating the striking decrease in vigor as the temperatures increase. The vigor of the roots correspond with that of the tops. At 15° C. roots were long and vigorous and became matted in the bottoms of the cans. At 20° they were only slightly less, but at 25° they were much reduced in length and fewer in number. At 30° they reached only about halfway to the bottom, and at 35° were only a few inches in total length. At 40° the plants did not survive.

Results in amount of root knot were possibly vitiated to a certain extent by the presence of the other organisms in the soil. They showed a striking increase with the temperature, however. Table 2 and the lettuce curve in Figure 7 show the marked effect of temperature on root-knot development with this crop. The same reservations on significance of results apply here as in experiment 2, because of the small number of individuals.

These results indicate that temperature is a limiting factor in root-knot development. They also indicate that in spite of the great differences in reaction of the hosts (tomato and lettuce) to temperature, the former being high-temperature-loving and the latter low, the results in root-knot infection were about the same. The need was evident for further experiments with a closer series of temperatures, and several different hosts.

EXPERIMENTS 4 AND 5

Using the same soil as before, increased in quantity by the addition of further compost and sand in the same proportions, and reinoculated with nematode material from Florida, a new series was started, as follows: Experiment 4 was conducted in a cool house, with six tanks, in which temperatures of 10°, 13°, 16°, 19°, 22°, and 25° C. were maintained. At the same time experiment 5 was started, in a warmer house, in seven tanks running 22°, 25°, 28°, 31°, 34°, 36°, and 38°. The plants used in the entire series were cucumbers and tobacco. Two cucumber plants were put into each of two cans in each tank, and two plants of White Burley tobacco into each of two more cans. The same series of temperatures was used for stem-nematode studies, so only the four cans were used for the root-knot organism. Since temperature and light conditions were markedly different in the two houses, the two experiments are considered as separate and distinct. No great fluctuations from the desired temperature or moisture occurred. The cucumbers did not grow well, possibly because of the season and shortage of light. In tank No. 1, at 10°, the first planting, and later a second one, succumbed completely to the unfavorable environment. Growth was increasingly better from 13° up to 25°. Root growth was fair, about 3 inches in depth, at 13°, and root knot was entirely absent. At 16° roots were somewhat better, and a small amount of root knot developed. At 19° root knot was abundant (figs. 8 and 9) on roots that extended in a few strands to the bottoms of the cans. At 22° roots and knots both were somewhat

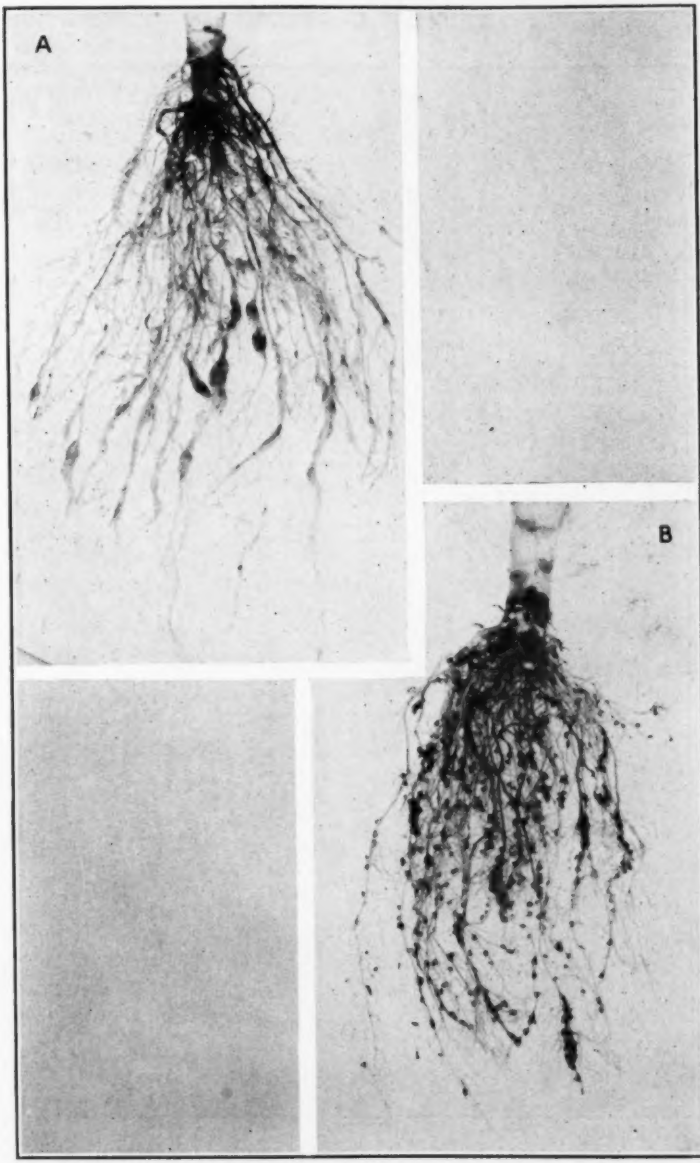

Fig. 8.—Roots of tobacco plants grown in root-knot-inoculated soil under controlled soil temperature conditions

A.—Root taken from a can held at 19° C., showing large galls.
B.—Root from the 25° C. tank, showing much more numerous, but smaller, galls, apparently burst out at the sides.

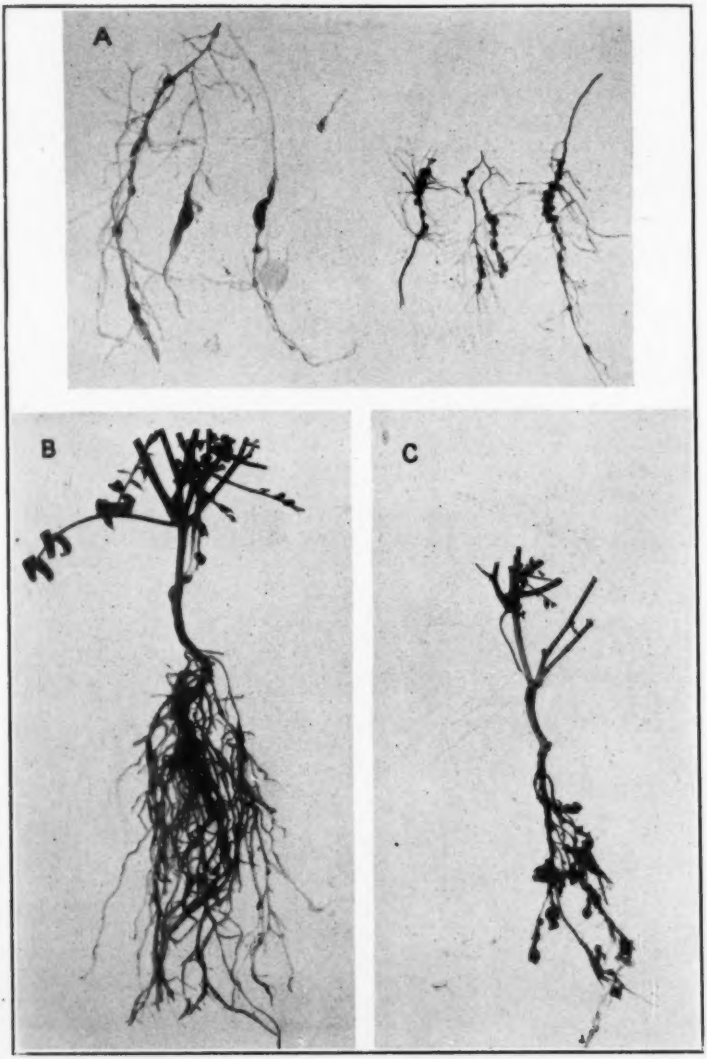

FIG. 9.—A.—Effect of temperature on type of root knots on tobacco: Left, Grown at 19° C.; Right, Grown at 25° C. B. and C.—Effect of temperature on development of root knot in vetch (*Vicia monantha*): B.—Plants grown at 12° C. Note the good nodule development, as well as the freedom from root knot. C.—Plants grown at 27° C. Note the poor root growth, abundant root knot, and total absence of beneficial nodules

reduced, but the amount of infection would appear to be an individual difference rather than a temperature reaction, for at the next higher temperature, 25°, the number of knots was double that of any lower temperature, and the roots were much reduced, due largely to the root knot. Table 3 shows the figures for amount of root knot, and Figure 10 shows the same results graphically.

The tobacco made much better growth than the cucumbers, and its reactions were such as to justify attributing them directly to temperatures. Figure 4, C, shows tobacco plants grown in tanks 1 to 6, and depicts clearly the relative growth at the different temperatures, there being a uniform increase from the low to high. Root-knot results were very striking, and, as shown in Table 3, bring out more sharply than does experiment 3 the critical temperature for development of the knots.

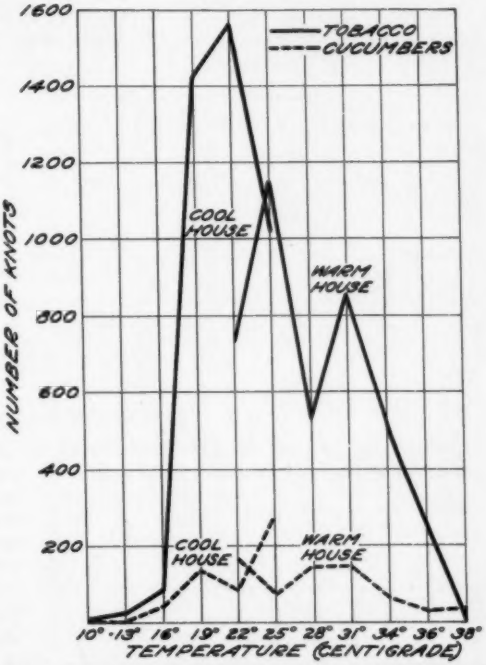

FIG. 10.—Effect of temperature on root-knot development in cucumber and tobacco

These experiments show that there is a critical temperature below which root knot does not develop. The differences in the tobacco plants between the averages at 16° and 19° C. are striking, and indicate that 16° or a trifle below that temperature is the dividing line between negligible infection and abundant and injurious infection. The infections at 10° and 13° may have taken place in the three-day interval before the cans were placed in the tanks, when they were all standing under equal conditions to get an even start; or, again, they may have taken place in the surface region of the soil; for, as has been said, in warm weather the surface layer sometimes warmed up a trifle in the lower tanks. It would appear to be justifiable to conclude, however, that the small amount of root-knot development at the lower temperatures was due to either the inactivity of the nematode at these temperatures, or to a host reaction that was common to both high and low temperature loving plants. Of the two explanations, the former seems the more plausible. At the other extreme, severity of root-knot infestation appears to be limited only by the

limited plant growth. There was a distinct dropping off in infection, however, in both cucumber and tobacco between 31° and 34° C. Above about 36° very little infection took place in the tobacco, though it was still abundant in cucumbers.

TABLE 3.—*Effects of soil temperature on root knot in cucumber and tobacco*

Tank No.	Temperatures, degrees C.	Cucumber (number of knots)	Tobacco (number of knots)		
			Plants in first series	Plants in second series	Average
COOL HOUSE					
1	10	0	0	8	4± 1.9
2	13	0	7	23	15± 3.8
3	16	39	60	98	79± 9.0
4	19	134	1,162	1,684	1,423±124.5
5	22	83	1,435	1,683	1,559± 59.1
6	25	270	628	1,423	1,026±189.3
WARM HOUSE					
1	22	165	339	1,125	732±187.4
2	25	71	1,056	1,266	1,161± 50.1
3	28	139	483	581	532± 23.4
4	31	142	1,138	563	851±137.4
5	34	61	391	651	521± 62
6	36	26	247	303	275± 13.4
7	38	31	----------	4	4

EXPERIMENT 6

In the latter part of February, 1922, an experiment was started with Early Buff soy beans. The cans were prepared as usual, and the bean seed planted, all the cans being held under the same conditions until they were well started. Approximately two weeks were required for this. On March 15 four inoculation and two control cans, each containing two soy-bean plants with the cotyledons fully developed into primary leaves, and secondary leaves just appearing, were placed into the tanks in a house averaging about 19° C. Soil moisture was maintained at 60 per cent of capacity. The temperatures of the tanks were started at 13°, 16°, 19°, 22°, 25°, and 28°, and held within a degree or two of these readings throughout the experiment, which closed April 6.

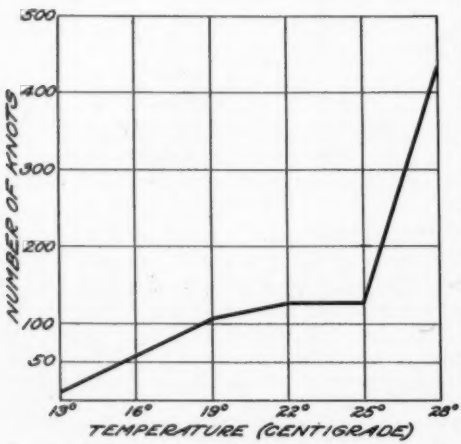

FIG. 11.—Effect of temperature on development of root knot in soy beans

The plants showed gradually increased vigor from the lowest to the highest temperatures. There was a distinct difference between the controls and the inoculated at the upper three temperatures, a slight difference at 19°, and none at all at the lower two. Root-knot development showed a distinct relation to temperature, as shown by Table 4 and Figure 11. Differences between adjacent temperature steps were sometimes not significant, but wider comparisons were usually distinctly so.

TABLE 4.—*Effect of soil temperature on root-knot development in soy beans*

Tank No.	Temperature, degrees C.	Control			Inoculated									
		Plants in first can	Plants in second can	Average height	Plants in first can		Plants in second can		Plants in third can		Plants in fourth can		Average	
		Height	Height		Height	Number of knots	Height	Number of knots	Height	Number of knots	Height	Number of knots	Height	Number of knots
		Cm.	*Cm.*	*Cm.*	*Cm.*		*Cm.*		*Cm.*		*Cm.*		*Cm.*	
1	13	12	12	12	9	4	10	10	10	6	10	3	10	6± 1
2	16	11	12	12	10	45	12	75	13	48	12	55	12	56± 4
3	19	14	13	13	12	56	13	153	11	76	12	141	12	106±14
4	22	16	14	15	13	225	13	31	13	128	14	117	13	125±23
5	25	18	18	18	16	133	15	86	15	226	18	55	16	125±22
6	28	22	23	22	19	186	18	669	21	502	19	321	19	420±61

EXPERIMENT 7

Early in the temperature studies it seemed desirable to conduct an experiment in which a controlled daily rise and fall of temperature could be compared with certain constant temperatures. Preliminary work at Madison, Wis., with a fluctuating-temperature tank made it evident that the exact range of temperatures desired could be obtained very closely. Consequently, at Washington, in the fall of 1922, the control apparatus was improved through one or two stages until the apparatus represented by the drawing in Figure 12 was made. *A* is the spring from an ordinary 5-cent mouse trap. The different parts are secured in place on a slab of transite by means of strong copper wire at *a*, *b*, *c*, and *d*. Points *a* and *b* are connected, by means of this same wire, with the binding posts *b* and *c*, respectively. Since the spring extension *e* must conduct an electric current, it is wound with copper wire of about 24 gauge, thus connecting posts *B* and *C* when the trap is set. The trip *f* is connected by means of a very fine wire with the clapper of an alarm clock.

A simple battery switch *FGH* is located near by. The wires from the heater are

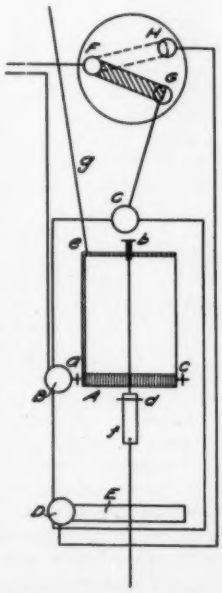

FIG. 12.—Diagram of the apparatus designed to control the temperature in a fluctuating-temperature tank

connected at point *F* on the switch, and at post *B* on the trap. *G* on the switch is connected by heavy current wire with post *C*, and *H* with post *D* on the trap. In the evening the switch connection *FH* is made, and the trap is set. The release of the alarm at the desired hour in the morning springs the trap. This makes the necessary contact on the copper plate (*E*) to close the circuit through the heater immersed in the tank. At the same time the pull of the spring is utilized to jerk out of its position by means of a string the outlet of the rubber hose carrying a stream of cold water, turning the water from the fluctuating tank to another outlet. The temperature of the tank immediately begins a gradual rise, therefore, until the desired upper limit is reached, when it is cut off by means of the usual thermostatic control.

Meanwhile, sometime during the day the trap and alarm are reset and the current switched from *FH* to *FG*. Now, when the trap is set the circuit is closed, instead of open as with the night setting; and when the trap is sprung at the desired hour in the afternoon, the contact made by the trap spring and tongue is broken, and the circuit is opened, thus immediately shutting off the heat in the tank. Again, the pull of the spring releases the small hose carrying the stream of water from its position during the day (where it has been held by a small plug in the pulley), so that it springs back and carries the stream into the fluctuating tank again. Thus the temperature immediately begins to fall, and continues to drop until the equilibrium of the city water temperature is reached. By running the stream of water fast or slow, as the need may be, a variation of two or three degrees in low temperature can be accomplished.

Experiment 7 was started on January 16, when three inoculation and one control cans each of lettuce and radish were planted. Three days later the cans were set into the tanks running temperatures as follows: No. 1, fluctuating, 10° to 19° C.; No. 2, 10°; No. 3, 15°; No. 4, 17°; No. 5, 19°; No. 6, 23°. Thermographic records were kept of water temperatures in the fluctuating, low, and medium temperatures by means of soil thermographs. Figure 13 shows specimen records for one week. Frequent tests showed that the soil temperature in the center of the cans followed very closely after the temperature of the water as indicated by the thermographs. The immersed bulb of the thermograph lagged somewhat behind the water itself, as did also the soil temperatures in the cans, so the record gives a fairly accurate representation of the changes that actually took place in the cans of soil. No great variations from the desired temperatures occurred in any of the other tanks. Moistures were kept up within five points of 60 per cent of the moisture-holding capacity of the soil.

It was not deemed profitable to make counts with the radishes, because of the unsuitability of the material for making this sort of record. The results from casual examination of the roots at the time they were removed showed that they followed very closely the results on the lettuce. No infection was evident at 10° C., a medium amount at 15° and the fluctuating temperatures, then an apparently increasing abundance at 17°, 20°, and 23°. The growth of the radishes was irregular, but apparently best at the medium and fluctuating temperatures, the roots being much better here than at any of the other temperatures.

The lettuce proved to be much the more desirable plant of the two for obtaining results on root knot. The response of the plants to the temperatures was somewhat as it was in experiment 3, though the higher temperatures were not nearly so hard on the plants, due to the fact that no injurious fungi were present in the soil. (Sterilized soil was used.) The plants grown at 15° C. and at the fluctuat-

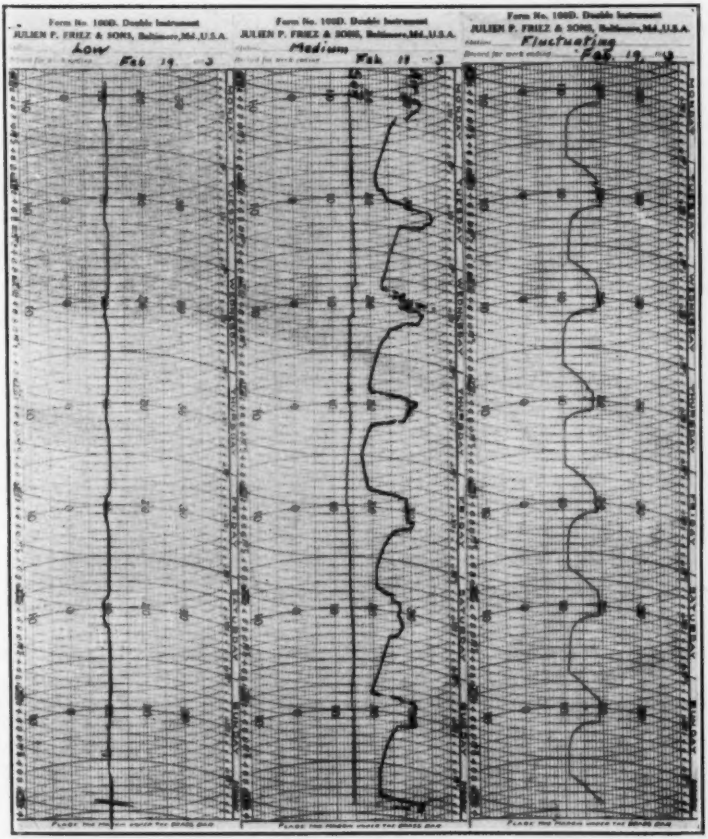
Form No. 100D. Double Instrument
JULIEN P. FRIEZ & SONS, Baltimore, Md., U.S.A.
Low
Feb 19 -3
Form No. 100D. Double Instrument
JULIEN P. FRIEZ & SONS, Baltimore, Md., U.S.A.
Medium
Feb 19 -3
Form No. 100D. Double Instrument
JULIEN P. FRIEZ & SONS, Baltimore, Md., U.S.A.
Fluctuating
Feb 19 -3

FIG. 13.—Record of temperatures in three of the tanks during one week of experiment 7, in the greenhouse at Washington, D. C. Note the fairly uniform daily range of temperature obtained in the fluctuating tank. The periodical drop indicated on the sheet marked "Low" was caused by the inflow of water from the fluctuating tank, when its temperature was automatically started on its rise. The middle sheet shows the air temperature, as well as the "Medium" soil temperature, for the week ending February 19

ing temperatures presented much the best appearance at the close of the experiment. At 10° good heads were produced, but they were not as large as those in the 15° tank. It is probable that the optimum for growth is between 10° and 15°. At the higher temperatures up to 20° good leaf growth was obtained, but the plants had long internodes and were not of good commercial type. At 23° the plants were smaller, showing that that was above the range of good growth.

Unfortunately, the soil used in this experiment had lost a great proportion of its nematodes when the last series (from experiment 2) were removed. Results therefore lack the striking contrasts that are usually present when the parasites are present in abundance. Table 5 indicates the effect of the temperatures on the lettuce root-knot infection. Figure 14 shows the same results.

TABLE 5.—*Effect of soil temperature on development of root knot in lettuce in three inoculation cans*

Tank No.	Temperature, degrees C.	Number of knots on—			
		Plants in first can	Plants in second can	Plants in third can	Average
1	10–19	55	145	45	82±17.5
2	10	0	6	0	2
3	15	150	72	94	105±12.8
4	17	450	167	220	279±47.8
5	20	610	380	367	452±43.4
6	23	218	245	231	231± 4.3

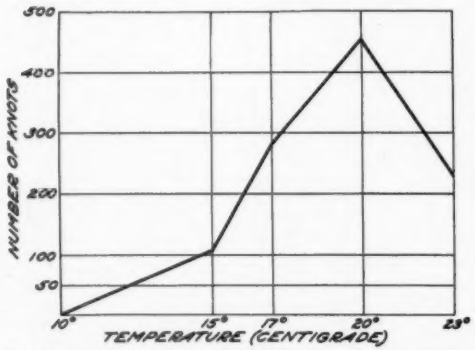

FIG. 14.—Effect of soil temperature on development of root knot in lettuce. The drop at 23° was due to reduced growth of the plant rather than to less favorable temperature

Table 5 and Figure 14 show practically no root knot at 10° C. and a greater amount with each rise in temperature up to 20°, as is to be expected from the results of previous experiments. The number of knots drops off at 23°, contrary to results with tobacco and tomato. This is to be expected, since it is in accord with the reduced growth of the host at that temperature. Root growth was much reduced, and number of knots per unit length was undoubtedly greater here than at any other temperature. These data, however, are entirely misleading if total number of knots alone is considered as the criterion of severity of root knot.

In a comparison of the root knot in the fluctuating tank with that at the average or 15° tank, the record of temperatures should be considered first of all. While the maximum reached was quite consistently 20° C., and the minimum 10°, making an average of 15°, certain irregularities resulted from differences in the rising and falling curves of temperature. These differences are plainly evident in Figure 9. Although the hours of beginning of the rise and fall were just 12 hours apart, careful computation showed that during the course of the experiment the temperature was above the average for 295 hours out of the total period of 938 hours, or only about 31.5 per cent of the time. In accordance with what might be expected from this, the number of knots at the fluctuating temperature is

somewhat less than the number at the average, the difference being 23. The probable error of the difference is 21.5. The chances are about even, then, that the difference is due to chance rather than temperature. The difference is therefore not significant.

Further study of the record shows that the temperature was above 13° C., the approximate critical temperature for noteworthy infection, for 411 hours, and below it for 527 hours. The period favorable for infection was undoubtedly long enough to overcome the quiescence due to the cooler temperatures, and to permit development of definite symptoms of the disease.

EXPERIMENT 8

In order to test this point still further, a final experiment with the same apparatus was started March 5. The soil was thoroughly mixed and reinoculated. Roots of French cocklebur containing an abundance of live nematodes were used as source of inoculum. Vigorous young celery plants that had been held in 2-inch pots until roots were abundant and tops were about 4 inches high were used. Uniform plants were set out, one to a can, six in inoculated and two in sterile control soil for each of the six tanks. Moisture was maintained at approximately 60 per cent of moisture-holding capacity of the soil. Plants were inserted into the tanks after three days, to become established under like conditions. Tank temperatures were adjusted to 11°, 14°, 17°, 20°, 23° C., and fluctuating from 11° to 18°. The automatic control for the latter tank was set to start the rise in temperature about 2 o'clock in the morning and the fall at 4 o'clock in the afternoon. This was designed to compensate for the difference in shape of the rising and falling curves, and thus to make the periods more nearly even above and below the critical temperature for infection.

The experiment was brought to a close April 4, just 30 days after it was started. Fairly uniform curves were obtained in the fluctuating-temperature tank. Occasional irregularities occurred. During the experiment the temperature was above 13° C. (the critical temperature for infection) for 361 hours, and below it for 359 hours. It was above that of the "mean" tank (14°) for 283 hours, and below for 437 hours. The temperatures in the constant-temperature tanks did not vary greatly from the desired points. The results are shown in Table 6, in total number of knots, and in number of knots per decimeter of root length. In order to arrive at an absolute basis of comparison, these results were studied statistically, the deviations of the different plants of one lot from the mean were recorded, and the standard deviation and the probable error of the mean were calculated.

With these figures at hand, the actual significance of any specific differences can readily be seen. Comparing first the constant temperatures, Figure 15 shows to best advantage the uniform rise in amount of infection from 0 in the 11° tank, to nearly 11 knots per decimeter of root length in the 23° tank. Between the 11° tank and the 14° tank significance is self-evident, and verifies earlier results as to the critical temperature. Between the 14° tank and the 17° tank the difference, 2.28 knots, is sufficiently greater than the probable error of the difference, 0.33, to be significant, consequently it is justifiable to conclude that even in this short range

the stimulation of increased temperature has made itself felt in increased amount of infection. This stimulation appears to be continuous up to the highest temperature tested, 23° C. Between the 20° and 23° tanks the difference is 4.83 knots per decimeter. The probable error of the difference is 0.93, or less than one-fifth the difference between the two, consequently the uniformity of the curve of infection may be judged to be significant.

TABLE 6.—*Effect of temperature on root knot in celery*

Tank and can number	Temperature	Length of root	Number of knots	Knots per dm.
Tank 1:	° C.	Cm.		
Can 1	11	1,727	0	0
Can 2	11	2,345	0	0
Can 3	11	2,196	0	0
Can 4	11	1,022	0	0
Can 5	11	1,988	0	0
Can 6	11	1,648	0	0
Can 1 (control)	11	1,816	0	0
Can 2 (control)	11	3,586	0	0
Tank 2:				
Can 1	14	1,401	312	2.227
Can 2	14	2,371	377	1.590
Can 3	14	1,889	411	2.175
Can 4	14	1,364	429	3.145
Can 5	14	1,898	560	2.950
Can 6	14	1,881	505	2.684
Can 1 (control)	14	1,823		
Can 2 (control)	14	3,228		
Mean				2.462±0.145
Tank 3:				
Can 1	17	874	379	4.336
Can 2	17	908	281	3.094
Can 3	17	1,203	503	4.181
Can 4	17	1,019	597	5.858
Can 5	17	1,785	842	4.717
Can 6	17	1,878	1,185	6.309
Can 1 (control)	17	1,270		
Can 2 (control)	17	4,992		
Mean				4.749±0.295
Tank 4:				
Can 1	20	1,021	619	6.062
Can 2	20	1,195	627	5.247
Can 3	20	2,197	1,804	8.211
Can 4	20	759	466	6.139
Can 5	20	895	502	5.608
Can 6	20	1,376	787	5.719
Can 1 (control)	20	443		
Can 2 (control)	20	6,431		
Mean				6.164±0.265
Tank 5:				
Can 1	23	1,024	1,270	12.402
Can 2	23	891	1,214	13.625
Can 3	23	665	927	13.939
Can 4	23	912	1,090	11.951
Can 5	23	488	219	4.487
Can 6	23	653	623	9.540
Can 1 (control)	23	1,126		
Can 2 (control)	23	1,309		
Mean				10.991±0.891
Tank 6:				
Can 1	[1] 11–23	4,823	157	.325
Can 2	[1] 11–23	2,242	314	1.400
Can 3	[1] 11–23	1,067	144	1.349
Can 4	[1] 11–23	3,364	85	.252
Can 5	[1] 11–23	1,029	150	1.457
Can 6	[1] 11–23	2,242	218	.972
Can 1 (control)	[1] 11–23	1,261		
Can 2 (control)	[1] 11–23	1,820		
Mean				.959±0.137

In comparing the plants grown in the fluctuating tank (12° to 18° C.) with those at 14° (just above the critical temperature), a significant difference is also observed, the difference of 1.49 knots being comparable with 0.20, a ratio of more than 7 to 1, which is well above the limits of what might be expected from random sampling. This is just as might be expected from the fact that the temperature was below 14° about 1.54 times as long as it was above, which shows again the rather delicate irritability of the nematodes to temperature as a stimulating agent. It shows that even several hours a day below the temperature at which they are capable of infesting a plant, does not render them incapable of infestation during the comparatively short period when temperature conditions are favorable.

EXPERIMENT 9

As a final experiment in the soil-temperature control series, tests were made with vetch, which is becoming more and more prominent as a winter green-manure crop in the South, and with potatoes. This series was started February 17, 1924, with rich sandy soil, steam-sterilized at first, then very heavily inoculated with fresh root-knot material of French cocklebur, *Urena lobata*, from Brooksville, Fla. In order to be certain of sufficient legume bacteria, a wet inoculation with a fresh culture of the vetch organism was made. The temperatures used were 12°, 15°, 18°, 21°, 24°, and 27° C.

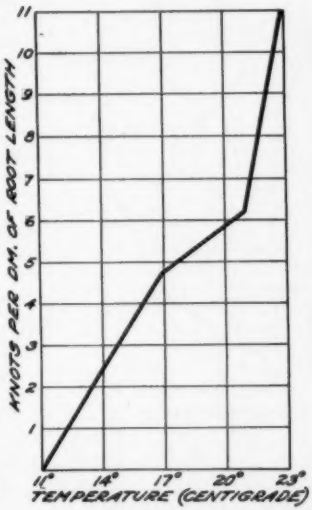

FIG. 15.—Effect of temperature on development of root knot in celery

The series was closed April 2. The temperature records, which were maintained throughout by means of soil thermographs, whose bulbs were immersed in the water at the average depth of the soil cans, showed in general very satisfactory control, except in the case of the low tank (12° C.), which occasionally ran too high. During the first week this tank was above 14°, the critical temperature for infection, for 10 hours. The third week it averaged more nearly 13° than 12°, and was above 14° for a total of 34 hours. In the fifth week it was up again on two occasions, for a total of 18 hours above 14°. The higher temperature was unavoidable; it was due to gradual rising of the temperature of the cold-water supply.

These 62 hours above the critical temperature for infection undoubtedly account for the infection that took place in the potato roots in this tank. While some of the other tanks, notably those at 18° and 21°, showed an occasional wider fluctuation than was desirable, the averages were approximately as anticipated.

The results of this experiment are given in Tables 7 and 8, and are shown in Figures 16 and 17. (See also fig. 9, B, C.) Here again statistical methods are followed in order to obtain a reliable basis for comparisons.

The results with the vetch are particularly noteworthy, and show clearly the effect of higher temperature in increasing the amount of root knot within a given period. Figure 16 shows the gradual and almost uniform rise from none at all at 12° C. to about 20 per decimeter of root length at 27°. The effect is almost the reverse for infection of the roots by the nodule bacteria. At the lower temperatures (12°, 15°, and 18°) nodule development was abundant. At 12° it was much less and at 24° and 27° it was practically negligible.

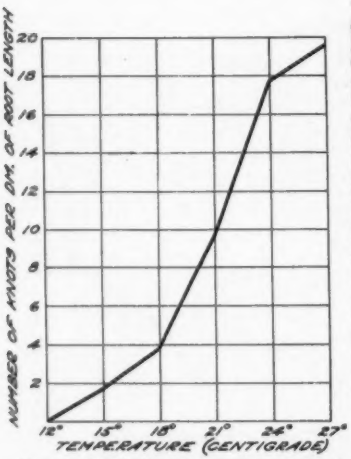

FIG. 16.—Effect of temperature on development of root knot in the vetch *Vicia monantha*

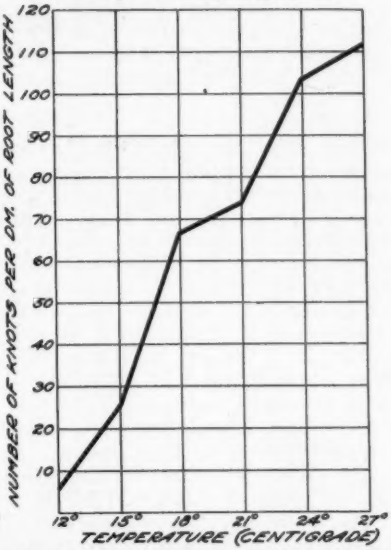

FIG. 17.—Effect of temperature on development of root knot in potatoes

The combined effects produced by temperature on the different growth factors were strikingly evident in the growth of the plants. At 12°, 15°, and 18° C. the plants were well supplied with beneficial nodules and were practically free from nematode root knots, and splendid growth occurred. Growth was greatly reduced at 24° and 27°, where root knot was abundant and beneficial nodules lacking. Figure 9, B, shows the root growth in the 12° tank, with good nodules and entire freedom from root knot. The plants grown at 15° were practically the same. Figure 9, C, shows the root of a plant grown at 27° C., with no nodules and abundance of root-knot infection.

With the potatoes the abundance of heavy root growth and the obvious uniformity of infection made it seems unnecessary to make complete counts of numbers of knots. Consequently, typical roots were selected from each plant and measured and number of knots counted. Estimates for the entire plant in number of knots per unit of length of root were made on this basis. The results as shown in the graph in Figure 17 agree with all previous results with other plants, in showing increase in infection with rise in temperature. This rise was particularly uniform with the potato.

TABLE 7.—*Effect of temperature on root-knot development in vetch*

Temperature and can No.	Length	Beneficial nodules	Knots	Knots per d. m.	Mean
12° C.:	*Cm.*		*Number*	*Number*	
Can 1	180	Clustered on upper part of main root and scattered throughout.	0		
Can 2	158	Scattered throughout root	0		
Can 3	168	One cluster on main root	0		
Can 4	143	3 small clusters	0		
Can 5	148	2 clusters	0		
15° C.:					
Can 1	133	Clustered on main root and scattered throughout.	17	1.278	
Can 2	136	Scattered	29	2.132	
Can 3	98	Scattered throughout	9	.918	
Can 4	152	A few scattered	30	1.973	
Can 5	146	Cluster on main root	29	1.986	1.657±.143
18° C.:					
Can 1	148	Few and scattered	42	2.84	
Can 2	93	Few	51	5.48	
Can 3	170	do	53	3.12	
Can 4	182	Cluster on main root	67	3.68	3.78±.31
Can 5					
21° C.:					
Can 1	107	Few	84	7.85	
Can 2	148	do	106	7.16	
Can 3					
Can 4	113	Different species, few nodules. Angustifolia.	167	14.78	
Can 5	52	None	44	8.46	9.56±.92
24° C.:					
Can 1	132	Few	114	8.64	
Can 2	40	Very few	52	13.00	
Can 3	145	Few, scattered	132	9.10	
Can 4	78	Very few	169	21.66	
Can 5	37	None	132	37.67	18.01±2.13
27° C.:					
Can 1	50	Two	110	22.00	
Can 2	67		155	23.13	
Can 3	28	Clustered about main root			
Can 4	41	Very few	83	20.24	
Can 5	87	do	116	13.33	19.68±1.25

TABLE 8.—*Effects of temperature on root-knot development in potato*

Temperature and can No.	Number of roots	Specimen root examined			
		Length	Number of knots	Number of knots per dm.	Mean
12° C:		*Cm.*			
Can 1	23	15	6	4.000	
Can 2	22	9	4	4.444	
Can 3	17	9	7	7.777	5.407±0.66
15° C.:					
Can 1	19	15	27	18.00	
Can 2	14	15	54	36.00	
Can 3	15	16	34	21.25	25.08+13.05
18° C.:					
Can 1	14	9	58	64.44	
Can 2	10	14	105	75.00	
Can 3	23	10	60	60.00	66.48±2.45
21° C.:					
Can 1	15	16	108	67.50	
Can 2	14	14	110	78.57	
Can 3	22	9.5	70	73.68	73.25±1.76
24° C.:					
Can 1	19	15	147	98.00	
Can 2	11	7	95	135.71	
Can 3	15	10	75	75.00	102.90±9.74
27° C.:					
Can 1	15	14	168	120.00	
Can 2	14	13	155	119.23	
Can 3	9	11	105	95.45	111.56±4.54

EFFECT OF TEMPERATURE ON THE PATHOLOGICAL ANATOMY OF THE HOST

Frequent observations during the recording of results of temperature-control experiments indicated marked differences in type of knot formed at the higher and the lower temperatures. This was best shown in the case of tobacco in experiment 4. At 25° C. the knots were not only more abundant than at 19°, but they were smaller, asymmetrical in shape, different in color, and indicated a difference in the relation of the parasite to the host plant. Close examination revealed that the nematodes were more frequently mature in the 25° tanks, and that dark masses on the sides of the galls were due to the extruded egg masses of the females. This stage in their maturity was reached before the galls had developed to the size of those in the 19° tank. The smaller, more mature galls were more injurious to the host than the others, for the difference between controls and inoculated plants was always greater at the higher than at the lower temperatures.

The same type of differences occurred with other crops as well, notably with celery and cucumber.

In experiment 9 with the potato, a peculiar warty formation in the vascular region of the cut surface of the seed piece, at the higher temperatures, was found to be due to infection of the seed piece. Mature female nematodes were found within such galls. This was observed in several different cases. The seed pieces had previously been freshly cut on one surface, the other side having become protected by a corky layer, which had developed as the seed piece lay in moss for sprouting. Only the freshly cut surface was found infested in the manner just described. In ordinary practice, potato seed-piece infection is not likely to be much of a factor in nematode increase, in view of the fact that at the time the potatoes are planted the temperature of the soil is ordinarily too low for much activity on the part of the eelworms.

CONCLUSIONS

General observations pointing toward climate as one of the limiting factors in the distribution of the root-knot nematode have been justified by a few definite experiments.

The results of the soil-temperature experiments are evident and conclusive. Below about 16° C. the amount of root knot is substantially less than it is only two or three degrees higher. Three degrees lower yet it is almost eliminated and is practically negligible. At 10° and 12° infections are very rare. Different crops vary to some extent in amount of infection at points near the critical temperature. Tobacco shows the change very abruptly, from the lower to a higher temperature, whereas with soy bean the change appears to be more gradual. With both, the critical temperature is much below the optimum for growth. With crops whose range of good growth includes the critical temperature, the difference in root-knot development are striking. At the higher temperatures root knot is abundant up to as high as the plants will grow. There appeared at times to be a dropping off of amount of the disease above 30°, but this was usually traceable to a decrease in available root growth, rather than an actual decrease in proportion of affected roots.

The similarity in effect on all the plants studied, high-temperature-loving and low-temperature-loving plants alike, lead to the belief that the reaction to temperature is on the part of the parasite rather than the host. Differences in type of knots are produced at different temperatures, to be sure, but here again at least a part of the difference is due to differences in stage of development of the nematode.

There appears to be opportunity for economic application of the results of investigations described in this paper. Many successful commercial greenhouse men throughout the country, as well as truck growers in the South, make use of the fact of the warmth-requiring proclivities of the root-knot nematode to grow successfully low-temperature-enduring plants, such as lettuce and celery, in root-knot-infested ground. Stone and Smith (*7*) reported that they had grown lettuce in soil following badly diseased cucumbers, and that root knot did not appear on it. They attributed this to resistance of the lettuce to nematode infection, and could not account for the fact that they had elsewhere seen lettuce badly knotted, unless by the possible occurrence of different strains of nematodes. It is probable that their lettuce was grown at the temperature most favorable to it, which is below the critical point for severe nematode infestation. Greenhouse managers may now be able to adjust temperatures with root-knot control in mind, with a possible saving in costs. Similarly, southern truck growers may be able to so adjust time of planting celery, lettuce, and other low-temperature-loving plants, as to avoid to a greater extent losses due to root knot.

The results of the experiment with vetch would seem to add greatly to the argument for the use of winter vetches as southern soil-improvement crops, in some sections at least. They should be valuable in root-knot control rotations. Even if susceptible to root knot, their principal period of growth would be below critical temperature for root-knot infection. As far south as Brooksville, Fla., soil temperature records show that for the winter of 1920–21 the soil temperature at 12 inches in depth was below the critical temperature for infection for only a short period, approximately a week in January. Prior to that it was below for parts of a day only. The use of a susceptible vetch in this locality, therefore, would not be practicable.

Farther north, however, and yet within the region of serious root-knot damage, lower soil temperatures are attained. At Monetta, S. C., the records for a 5-inch depth show an almost constant temperature below 13° C. from about the 1st of December, 1921, to about the middle of April, 1922. At 12 inches it was approximately the same, with less fluctuation and a slower response to spring warmth. At this place, therefore, a hardy fall-planted vetch grows readily until well along in the spring, without risk of increasing the nematodes, and with definite soil improvement.

The amount of moisture seems to play only a small part in root-knot development, so long as the moisture content of the soil is favorable to the growth of the crops. Within the range of 40 to 80 per cent of the moisture-holding capacity of the soil there is very little difference in root-knot development. At 60, 70, and 80 per cent there appears to be a slight increase over the other percentages. Even below 40 per cent, which is too dry for good growth of ordinary crops, and above 80, which is more or less muddy, considerable root knot occurs.

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A STUDY OF SOIL HETEROGENEITY IN EXPERIMENT PLOTS[1]

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INTRODUCTION

In carrying on plot experiments the agronomist is continually confronted with the problem of soil heterogeneity, a problem so important that no field experiments are carefully planned without considering it. Several ways of overcoming the effect of soil heterogeneity have been suggested, one of which is replication of plots in such manner as to take into account the uniformity of the soil as revealed by production.

A few years ago the West Virginia Agricultural Experiment Station acquired about 16 acres of second-bottom land in Mason County near the Ohio River at Maggie, W. Va. The tract is level and to the eye appears quite uniform. It is mapped as "Wheeling fine sandy loam" by Bureau of Soils [2] of the United States Department of Agriculture. The greater part of the tract was available for rotation experiments, but before beginning the experiments it was decided to make a soil-uniformity test by growing blanket crops for two successive years.

PLAN OF THE FIELD

In all, 270 plots, each 68 feet by 21 feet, were laid off for the rotation experiments. Although the gross area of a plot was approximately $\frac{2}{61}$ of an acre, yield was based on an area 61 feet by 14 feet only. A border 3½ feet wide on the sides and ends of each plot was discarded at harvest, the area upon which yield was based being approximately $\frac{1}{61}$ of an acre. The general plan of the field experiment is shown in Figure 1.

The plots were laid out in three double series, with a 14-foot roadway around the entire field and between the double series. The plot numbers, and the plots used for a certain rotation, are given in the diagram, the latter being indicated by letter. The letter a is found in plots 1, 13, 25, and 33, and a′ is found in plots 111, 123, 135, and 143. The crop rotation for the first series of plots is corn first year, wheat second year, clover and timothy third year, and potatoes fourth year, with a cover crop between certain crops. The number of plots and the number of years to complete the rotation were arranged to correspond, so that each crop would be grown each year. This cropping system is duplicated in plots 111, 123, 135, and 143, designated by a′. For example, in 1925 plots 1 and 111 were occupied by corn, 13 and 123 by wheat, 25 and 135 by clover and timothy,

[1] Received for publication Dec. 28, 1925; issued August, 1926. Approved for publication by the Director of the Agricultural Experiment Station of West Virginia University as Scientific Paper No. 11.

[2] LATIMER, W. J., and MOONEY, C. N. SOIL SURVEY OF THE POINT PLEASANT AREA, WEST VIRGINIA. U. S. Dept. Agr., Bur. Soils, Field Oper. 1910, Rpt. 14: 1077–1122, illus. 1912.

and 33 and 143 by potatoes. The scheme of replication for the other rotations is indicated in similar manner—a certain letter indicates a given rotation, and the same letter primed indicates the duplicate plots.

Roadway	Plot	Plot	Roadway	Plot	Plot	Roadway	Plot	Plot	Roadway
	Roadway								
	1 a	51		101 s	151		201 m′	251	
	2 b	52		102 t	152		202 n′	252	
	3 c	53		103 q	153		203 o′	253	
	4 d	54		104 v	154		204 m′	254	
	5 e	55		105 r	155		205 n′	255	
	6 f	56		106 q	156		206 o′	256	
	7 g	57		107 q	157		207 m′	257	
	8 h	58		108 u	158		208 n′	258	
	9 i	59		109 v	159		209 m′	259	
	10 j	60		110 x	160		210 w′	260	
	11 k	61		111 a′	161		211 p	261	
	12 l	62		112 b′	162		212 p	262	
	13 a	63		113 c′	163		213 p	263	
	14 b	64		114 d′	164		214 p	264	
	15 c	65		115 e′	165		215 q	265	
	16 d	66		116 f′	166		216 r	266	
	17 e	67		117 g′	167		217 s	267	
	18 f	68		118 h′	168		218 t	268	
	19 g	69		119 i′	169		219 u	269	
	20 h	70		120 j′	170		220 q	270	
	21 i	71		121 k′	171		221 r	271	
	22 j	72		122 l′	172		222 s	272	
	23 k	73		123 a′	173		223 t	273	
	24 l	74		124 b′	174		224 q	274	
	25 a	75		125 c′	175		225 s′	275	
	26 b	76		126 d′	176		226 r	276	
	27 c	77		127 e′	177		227 t′	277	
	28 d	78		128 f′	178		228 q′	278	
	29 e	79		129 g′	179		229 u′	279	
	30 f	80		130 h′	180		230 r′	280	
	31 g	81		131 i′	181		231 q′	281	
	32 h	82		132 j′	182		232 q′	282	
	33 a	83		133 k′	183		233 q′	283	
	34 b	84		134 l′	184		234 r′	284	
	35 c	85		135 a′	185		235 s′	285	
	36 w	86		136 b′	186		236 t′	286	
	37 m	87		137 c′	187		237 u′	287	
	38 n	88		138 d′	188		238 q′	288	
	39 o	89		139 e′	189		239 r′	289	
	40 m	90		140 f′	190		240 v′	290	
	41 n	91		141 g′	191		241 t′	291	
	42 o	92		142 h′	192		242 q′	292	
	43 m	93		143 a′	193		243 r′	293	
	44 n	94		144 b′	194		244 s′	294	
	45 m	95		145 c′	195		245 v′	295	
	Roadway								

FIG. 1.—Field plan of the plots used in the rotation experiments at Maggie, W. Va.

In Figure 1 letters are given to only the first series of each double eries of plots. The system of cropping in the second series was imilar to the first series, and the manner of replication in the two series exactly corresponded. The difference between the first and second members of each double series is a matter of lime treatment, of cover crops, or of handling crop residues. The duplicates of plots 51, 63, 75, and 83 are 161, 173, 185, and 193, respectively. In similar manner the cropping system and the method of replication with respect to the other crop rotations in the second series follows that of the first series.

THE CROPS AND METHODS OF DETERMINING YIELDS

Before actually beginning the rotation experiments, and before any differential treatment was given to the plots, the whole area diagrammed in Figure 1 was cropped first (in 1923) to oats, and then (in 1924) to winter wheat. Fulghum oats and Gladden wheat were used. Both crops were seeded with a grain drill, and the rows were run lengthwise of the plots. The oats was seeded at the rate of approximately 2½ bushels per acre, and the wheat at about 6 pecks per acre. The oats was harvested for hay and the wheat for grain. The crops on the borders of each plot were removed before harvest, and in the case of oats the hay produced on the entire net area (61 by 14 feet) of each plot was weighed. At the same time a sample of approximately 500 gm. of hay was taken and carefully weighed. These samples were hung in a well-ventilated shed, and when they reached a relatively constant weight they were weighed again. The second weighing was on a dry, sunshiny day. The amount of air-dry material produced per plot was computed from the data so obtained.

The yield of wheat was determined in the manner suggested by Arny and Garber.[3] There were no facilities for threshing the grain of each plot separately, so the yields were determined from five rod rows from each plot. One drill row 16 feet long was removed from the corner of each plot (after borders had been removed), and one drill row of the same length was taken from the middle of the plot. No attempt was made to keep the rod rows of any one plot separate. This material in bundles was hung in a shed until dry and was then weighed. After the bundles were weighed the spikes were removed from the straw and shipped to Morgantown, where they were threshed and the yield of grain determined. The yield in bushels per acre of each plot was calculated from these results. The yield of the straw was obtained by subtracting the weight of the grain from the total weight of the rod-row bundles.

After the rod rows were removed each plot was harvested separately with a grain binder. The bundles were tagged and set up in small shocks and covered with canvas. After the shocks had thoroughly dried out under these conditions the bundles from each plot were weighed. In this way the yield of grain plus straw was determined.

YIELDS BASED ON ROD-ROW SAMPLES

Before discussing the plot yields as a measure of soil uniformity it may be well to present certain data obtained in 1924 in connection with determining the wheat yields. As has been stated, the yields of wheat in bushels per acre were based on five rod rows taken from each plot. The weight of the straw of the rod-row samples was determined by subtracting the weight of the threshed grain from the total weight. In addition to these data, the weight of the air-dry wheat bundles (straw plus grain) remaining on each plot after the rod rows were removed was determined. By adding this weight to the weight of the rod-row bundles the total production of grain and straw on each plot was ascertained. The interrelation of these

[3] ARNY, A. C., and GARBER, R. J. FIELD TECHNIC IN DETERMINING YIELDS OF PLOTS OF GRAIN BY THE ROD-ROW METHOD. Jour. Amer. Soc. Agron. 11: 33–47, illus. 1919.

data, as well as the relation between these data and the computed yield of grain for the entire plot, was studied. By means of the ratio of grain to straw from the rod rows, and the total weight of the grain plus the straw from the entire plot, the yield of the grain on each entire plot was computed.

The correlation between the yield of grain and the yield of grain plus straw, all based on the rod-row samples, is shown in Table 1. The table shows a marked positive correlation ($+0.951\pm0.004$) between these two sets of variables. The regression equation for yield of grain on yield of grain plus straw is $Y_g=-0.505+5.478X_{g+s}$. The standard error of estimate, as determined by the usual formula ($\sigma y.x=\sigma y\sqrt{1-r^2}$), is 1.207, or about 30.9 per cent of the standard deviation of the yield of grain. These facts show that in this experiment one may predict with a fair degree of precision the yield of grain from the yield of grain plus straw.

TABLE 1.—*Correlation of yield of grain from five rod rows with yield of grain plus straw from five rod rows at Maggie, W. Va.*

	Grain plus straw, pounds															
	1.5	1.8	2.1	2.4	2.7	3	3.3	3.6	3.9	4.2	4.5	4.8	5.1	5.4	5.7	
Grain, bushels per acre:																
7.5	1	1														2
9		4	3	0												7
10.5		3	16	4	1											24
12			10	21	3											34
13.5				13	17	1										31
15				1	31	19										51
16.5						25	17	1								43
18					1	4	22	7								34
19.5				1	1		2	8	3							15
21								5	10	1		1				17
22.5									1	0						1
24									1	2						3
25.5											1	1				2
27													1	1		2
28.5													1			1
30													1	1	1	3
	1	8	29	40	54	49	41	21	15	3	1	2	3	2	1	270

$r=+0.951\pm0.004$.

In view of the foregoing observations, and if the five rod rows from each plot are representative of the plot, a marked positive correlation would be expected between the yield of grain based on the rod-row samples and the yield of the total grain plus straw based on the entire plot. This expectation was fulfilled, as is shown in Table 2. Although the correlation coefficient (0.841 ± 0.012) obtained is not as high as the one just discussed above, it nevertheless shows a marked positive correlation between the two sets of variables. The regression equation for yield of total grain plus straw on yield of grain from the rod rows is $y_{g+s}=13.147+2.269\times_g$. The standard error of the estimated yields of grain plus straw by means of the regression equation is 5.699, or about 54.1 per cent of the standard deviation of the yields of grain plus straw. It is obvious that the predicted values from this equation are considerably less trustworthy than the predicted yields of grain from the yield of grain plus straw all based on the rod-row samples.

TABLE 2.—*Correlation of yield of grain plus straw from entire plot with yield of grain from five rod rows at Maggie, W. Va.*

Grain plus straw, pounds (entire plot):	Grain bushels per acre (rod rows)																
	7.5	9	10.5	12	13.5	15	16.5	18	19.5	21	22.5	24	25.5	27	28.5	30	
22.5		1															1
27.5	2	2	2	1													7
32.5		2	7	2			1										12
37.5		1	10	12	4	3											30
42.5		1	3	11	14	15	2	2	2								50
47.5			2	6	9	18	17	9	2								63
52.5				1	3	13	12	12	4	3							48
57.5				1	1	1	6	9	5	5							28
62.5						1	4	2	2	5	1	1					16
67.5							1			3		1	1				6
72.5												1	1				2
77.5														1			1
82.5										1				1	1	2	5
87.5																1	1
	2	7	24	34	31	51	43	34	15	17	1	3	2	2	1	3	270

$r = +0.841 \pm 0.012$

In addition to the two coefficients of correlation mentioned certain others were calculated. The correlation coefficient obtained for the yield of grain plus straw from the rod rows and the yield of grain plus straw from the entire plot was $+0.872 \pm 0.010$; for the yield of grain from the rod rows and the computed yield of grain from the entire plot, $+0.894 \pm 0.008$; for the computed yield of grain from the entire plot and the yield of grain plus straw from the entire plot, $+0.940 \pm 0.005$; and for the yield of grain plus straw from the rod rows and the computed yield of grain from the entire plots, $+0.844 \pm 0.012$. In all cases the coefficients show a marked positive correlation. Of particular interest is the high correlation between the yield of the grain plus straw from the rod rows and the total yield of the grain plus straw from the entire plot. This high correlation shows that the weight of the rod-row samples (grain plus straw) from each plot is a fairly reliable index to the total weight of the grain plus straw produced on that plot. The high positive correlations obtained between the computed yields of the entire plots and the experimentally determined yields of the plots are expected, in view of the high correlations found among the latter.

It is believed that the facts brought out above justify the use of rod-row samples in determining yield. The discussion of plot uniformity which follows later is based on the yield of wheat in bushels per acre as determined from the rod-row samples removed from each plot.

PLOT UNIFORMITY AS SHOWN BY YIELDS OF OAT HAY

The yield of air-dry oat hay in pounds per acre is shown in Table 3. The mean yield of all the plots is 1,883.7 pounds. It will be observed that the yield of each plot is expressed in the table as a deviation from this mean yield. If the yield of a particular plot is less than the mean yield it is indicated as a minus deviation; on the other hand, if the yield of a plot is greater than the mean yield it is shown as a plus deviation.

TABLE 3.—*Yield of oat hay in pounds of air-dry material per acre per plot, expressed as deviations from the mean yield of all the plots in the crop-rotation experiments at Maggie, W. Va. Yield based on the production of the net area of each plot in 1923*

Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot
1	+39.3	+182.3	51	101	+59.3	+258.3	151	201	+39.3	+80.3	251
2	−37.7	+59.3	52	102	+39.3	+171.3	152	202	−123.7	+136.3	252
3	+23.3	+212.3	53	103	−67.7	−32.7	153	203	−1.7	+243.3	253
4	−52.7	+161.3	54	104	−103.7	+80.3	154	204	−154.7	+406.3	254
5	−32.7	+493.3	55	105	+666.3	+717.3	155	205	+345.3	+758.3	255
6	+34.3	+253.3	56	106	+880.3	+952.3	156	206	+273.3	+431.3	256
7	+207.3	+426.3	57	107	+896.3	+839.3	157	207	+304.3	+105.3	257
8	+324.3	+294.3	58	108	+493.3	+391.3	158	208	+396.3	+74.3	258
9	+29.3	+18.3	59	109	+340.3	+350.3	159	209	+187.3	+29.3	259
10	+156.3	+248.3	60	110	+176.3	+23.3	160	210	+309.3	+360.3	260
11	−333.7	−67.7	61	111	+278.3	−174.7	161	211	−72.7	+1,523.3	261
12	−108.7	−78.7	62	112	−62.7	−108.7	162	212	+64.3	+2,650.3	262
13	+8.3	−123.7	63	113	−256.7	−149.7	163	213	−16.7	+2,655.3	263
14	−42.7	+29.3	64	114	−241.7	−164.7	164	214	−16.7	+1,100.3	264
15	+238.3	−1.7	65	115	−445.7	−246.7	165	215	−123.7	+156.3	265
16	+136.3	+299.3	66	116	−220.7	−108.7	166	216	−159.7	+23.3	266
17	+8.3	−185.7	67	117	−480.7	−83.7	167	217	−62.7	−88.7	267
18	−282.7	−190.7	68	118	−169.7	−419.7	168	218	−231.7	−144.7	268
19	−246.7	−88.7	69	119	−256.7	−88.7	169	219	−220.7	−78.7	269
20	−382.7	−215.7	70	120	−266.7	−180.7	170	220	−169.7	+105.3	270
21	−266.7	−195.7	71	121	−195.7	−180.7	171	221	−139.7	−1.7	271
22	−419.7	−164.7	72	122	−93.7	−1.7	172	222	+59.7	−16.7	272
23	+8.3	+49.3	73	123	−210.7	−180.7	173	223	−134.7	+23.3	273
24	−266.7	−174.7	74	124	−287.7	−266.7	174	224	−378.7	−103.7	274
25	−307.7	−322.7	75	125	−292.7	+3.3	175	225	−282.7	−134.7	275
26	+13.3	−338.7	76	126	−256.7	−246.7	176	226	−261.7	−327.7	276
27	−292.7	−256.7	77	127	−67.7	−159.7	177	227	−231.7	−246.7	277
28	−363.7	−317.7	78	128	−251.7	−210.7	178	228	−220.7	−338.7	278
29	−282.7	−424.7	79	129	−307.7	−190.7	179	229	−419.7	−470.7	279
30	−215.7	+23.3	80	130	−88.7	−154.7	180	230	−210.7	−205.7	280
31	+748.3	+59.3	81	131	+238.3	−200.7	181	231	−210.7	−47.7	281
32	+549.3	+340.3	82	132	+13.3	−83.7	182	232	−139.7	−72.7	282
33	+656.3	+166.3	83	133	−134.7	−180.7	183	233	−129.7	−47.7	283
34	−93.7	−149.7	84	134	−271.7	−62.7	184	234	−190.7	+248.3	284
35	+156.3	+105.3	85	135	−220.7	−333.7	185	235	−348.7	−83.7	285
36	+258.3	+258.3	86	136	−67.7	−1.7	186	236	−271.7	−118.7	286
37	+131.3	+253.3	87	137	+115.3	+125.3	187	237	−266.7	+29.3	287
38	+100.3	+182.3	88	138	+396.3	+202.3	188	238	−465.7	−358.7	288
39	+80.3	+156.3	89	139	+314.3	+574.3	189	239	−225.7	−195.7	289
40	+141.3	+23.3	90	140	+488.3	+34.3	190	240	−741.7	−317.7	290
41	+324.3	+340.3	91	141	+564.3	−88.7	191	241	−674.7	−598.7	291
42	+131.3	−353.7	92	142	−88.7	−169.7	192	242	−368.7	−307.7	292
43	+49.3	−251.7	93	143	−802.7	−72.7	193	243	−669.7	−169.7	293
44	−11.7	−159.7	94	144	−572.7	+207.3	194	244	−180.7	+64.3	294
45	−185.7	−205.7	95	145	−287.7	+64.3	195	245	+187.3	−190.7	295

Mean=1,883.7 pounds.

The correlation of yields between contiguous plots, as suggested by Harris,[4] was calculated, the magnitude of the correlation so obtained indicating the degree of the soil heterogeneity of this field. If the field is "patchy," the yields of contiguous plots will tend to be of the same order and therefore a high correlation will be obtained.

In applying the formula [5] suggested by Harris the plots were first grouped in a 2×2-fold manner.

[4] HARRIS, J. A. ON A CRITERION OF SUBSTRATUM HOMOGENEITY (OR HETEROGENEITY) IN FIELD EXPERIMENTS. Amer. Nat. 49: 430–454, illus. 1915.

(5) $$r_{p_1p_2}=\frac{\{[S(C^2p-S(p^2)]/m[n(n-1)]\}-\bar{p}^2}{\sigma p^2}$$

when S indicates summation, p the yield of the ultimate plots, Cp the yield of the combination plots grouped in a certain manner, n the number of ultimate plots in each combination plot, m the number of combination plots, $\bar{p}$ the average yield of the ultimate plots, and σp their standard deviation.

For example, plots 1, 2, 51, and 52 were grouped together, and 3, 4, 53, and 54 were grouped together, and the other plots were grouped in a similar way, with the following exceptions: Plots 45, 95, 245, and 295 were placed in one group and 109, 159, 111, and 161 in another group. The first exception was made to take care of the last pair of plots in the first and third double series, respectively. The reason for the other exception is explained below.

Plots 211 to 214, and 261 to 264, inclusive, were eliminated from this study because of the fact that a few years ago a straw stack had stood on or in the vicinity of plot 263, which undoubtedly accounts for the relatively high yields on plots 261 to 264, inclusive. Plots 110 and 160 were eliminated because in the rotation experiments they were to be used for a continuous cropping study and were not to be replicated.

The plots were also grouped in a 3×2-fold manner—for example, plots 1, 2, 3 were grouped together, and plots 51, 52, and 53 were grouped together, etc.—and the coefficients of correlation were calculated. The irregularity in the 3×2-fold grouping occurred with plots 144, 145, 194, 195, 245, and 295, which were grouped together. In addition to the plots already mentioned, plots 210 and 260 also were eliminated from consideration in the 3×2-fold groups.

After calculating the two coefficients of correlations for yields of contiguous plots in the manner mentioned above, a third correlation coefficient was calculated by means of the formula suggested by Harris for the yields of replicated plots. The plots were placed in groups of fours made up according to the replications which it was proposed to use in the rotation experiments. (See fig. 1.) For example, plots 1, 51, 111, and 161 were placed in one group, plots 2, 52, 112, and 162 were placed in another group, and similarly each pair of plots (end to end) was grouped with its proposed replicate in the rotation experiments.

In addition to testing for soil uniformity by Harris's method, one may obtain a measure of uniformity by correlating in the usual way the yields of contiguous plots. This was done for the yields of the plots under consideration. First a correlation table was prepared correlating the yields of plot 1 with 2, 2 with 3, 3 with 4, and proceeding in this way to the end of the series (plot 45) and then beginning at plot 51 and correlating it with 52, 52 with 53, etc. In this manner a correlation table was prepared which included all the plots except those eliminated, namely, 110, 160, 211, 212, 213, 214, 261, 262, 263, and 264. Where the continuity of a plot series was broken on account of the elimination of certain plots, the plots immediately above and below the "break" were treated the same as the last and first plot, respectively, of a series. In a somewhat similar manner a correlation table was prepared, using the yield of each plot but once. For example, the yield of plot 1 was correlated with the yield of plot 2, plot 3 with 4, plot 5 with 6, etc., proceeding in a regular manner from top to bottom of each series, except in the cases where plots were eliminated. To distinguish the two correlation coefficients so obtained, the first is designated as "all contiguous plots" and the second as "paired contiguous plots."

To further test the effectiveness of the proposed manner of replication in overcoming soil heterogeneity, still another correlation table was prepared in which the yield of each plot was correlated

with the yield of its replicate. For example, the yield of plot 1 was correlated with the yield of plot 111, 51 with 161, 2 with 112, 52 with 162, and so on for the rest of the plots in the rotation experiments. The correlation coefficient so obtained is designated as "paired replicated plots."

The correlation coefficients obtained by Harris's method, together with those calculated in the usual manner, are given in Table 4. The coefficients obtained by the two methods agree, in that they show a rather marked soil heterogeneity for the experimental field under observation. Also, the particular manner of replication was helpful in overcoming the objectionable effect of soil heterogeneity.

TABLE 4.—*Coefficients of correlation between yields of oat hay grown on certain plots at Maggie, W. Va., in 1923*

Method of calculation	n	r
Harris's 2×2-fold contiguous	260	+0.631±0.025
Harris's 2×3-fold contiguous	258	+.542±.030
Harris's 2×2-fold replicated	260	+.134±.041
Ordinary "all contiguous plots"	250	+.668±.024
Ordinary "paired contiguous plots"	130	+.694±.031
Ordinary "paired replicated plots"	130	−.068+.059

The correlation coefficient obtained by applying Harris's formula to the yields of contiguous plots, are, for the 2×2-fold grouping +0.631±0.025, and for the 2×3-fold grouping +0.542±0.030; the difference between the coefficients is 0.089±0.039, a difference which is only slightly more than two times its probable error. (All probable errors of differences were calculated by the approximate formula $D_e=\sqrt{e^2_1+e^2_2}$.) When Harris's formula is applied to replicated plots grouped in a 2×2-fold manner, a correlation coefficient of +0.134±0.041 is obtained which is significantly less than either of the coefficients obtained by grouping contiguous plots. This constant is approximately 3.3 times its probable error, and shows that there is only a slight correlation between the yields of replicated plots.

The correlation coefficient obtained by the ordinary method for "all contiguous plots" is +0.668±0.024, and for "paired contiguous plots" +0.694±0.031. These coefficients do not differ significantly from one another, and neither does either of them differ significantly from the coefficient obtained by Harris's formula applied to the yields of contiguous plots grouped in a 2×2-fold manner. The correlation coefficient obtained by the ordinary method for "paired replicated plots" is −0.068±0.059. The difference between this coefficient and the one obtained for replicated plots by Harris's method is 0.202±0.072, a difference which although it is considerable, is slightly less than three times its probable error.

PLOT UNIFORMITY AS SHOWN BY YIELDS OF WHEAT

The yields of wheat in bushels per acre based on the five rod rows removed from each plot are given in Table 5. As in the case of oat hay, the yields of wheat are expressed as deviations from the mean yield of all the plots. The yield of any particular plot is easily ascertained by adding or substracting (as the case may be) the deviation from the mean yield (15.6 bushels).

TABLE 5.—*Yield of wheat in bushels per acre per plot, expressed as deviations from the mean yield of all the plots in the crop-rotation experiments at Maggie, W. Va. Yield based on the average production of five rod rows removed from each plot at harvest in 1924*

Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot	Plot	Dev.	Dev.	Plot
1	−0.5	+1.4	51	101	+2.9	−1.0	151	201	−5.2	−4.0	251
2	−6.0	+1.7	52	102	+0.2	−6.0	152	202	−6.6	−4.7	252
3	−0.6	−0.3	53	103	−4.1	−5.0	153	203	−6.7	−4.6	253
4	−3.1	−2.4	54	104	−7.0	−5.3	154	204	−7.8	−1.2	254
5	−1.3	−5.0	55	105	−1.3	−2.5	155	205	−7.7	−2.0	255
6	+1.7	−3.8	56	106	−1.4	+0.4	156	206	−3.9	−4.1	256
7	−0.1	+1.8	57	107	+6.4	−0.5	157	207	−5.6	−4.2	257
8	−2.2	+1.6	58	108	−2.0	−3.3	158	208	−1.2	−3.4	258
9	+3.5	+3.5	59	109	−2.9	−5.2	159	209	−4.1	−3.0	259
10	+3.5	+2.7	60	110	−5.8	−4.0	160	210	−4.3	−0.9	260
11	−4.9	−2.1	61	111	−0.6	−3.3	161	211	−4.7	+5.0	261
12	+0.7	−3.7	62	112	−2.4	−0.8	162	212	−1.3	+14.1	262
13	+0.9	−1.4	63	113	−1.2	+0.1	163	213	+0.7	+15.0	263
14	+1.0	+0.4	64	114	−5.3	−1.6	164	214	+2.0	+5.7	264
15	+4.0	+3.1	65	115	−0.8	+1.0	165	215	+0.1	+1.1	265
16	+2.8	+2.3	66	116	−1.6	−1.3	166	216	−3.6	−0.7	266
17	+0.4	+0.2	67	117	+2.2	−0.6	167	217	−1.0	+1.7	267
18	+0.6	−1.0	68	118	−0.3	−3.4	168	218	+0.7	−1.2	268
19	−4.8	−2.3	69	119	+1.8	+4.9	169	219	+0.5	−0.7	269
20	−4.7	−4.9	70	120	+1.2	−0.9	170	220	−2.7	+1.9	270
21	−5.2	−4.4	71	121	+2.7	+4.7	171	221	−0.3	+3.0	271
22	−2.1	−3.9	72	122	−1.9	+4.0	172	222	+3.6	+2.1	272
23	+2.6	−5.4	73	123	−0.8	−0.1	173	223	−2.9	−0.9	273
24	−3.5	−5.9	74	124	+5.2	+3.7	174	224	+4.8	+2.3	274
25	−2.0	−4.2	75	125	−1.0	+2.6	175	225	−1.0	+5.5	275
26	−0.8	+0.5	76	126	+3.5	+2.2	176	226	+3.8	+2.4	276
27	+1.9	−1.9	77	127	−1.0	+0.6	177	227	+1.0	−2.7	277
28	+1.2	+1.5	78	128	+0.1	−5.9	178	228	−1.7	−0.5	278
29	+4.1	−2.9	79	129	+0.8	−2.2	179	229	−1.9	−4.0	279
30	+1.7	−3.8	80	130	−1.2	+2.4	180	230	−4.9	+1.3	280
31	+11.1	+3.0	81	131	+1.7	+0.5	181	231	−1.7	+0.2	281
32	+10.9	+5.4	82	132	+2.9	+1.6	182	232	−3.0	−0.4	282
33	+10.5	+1.6	83	133	−0.7	+1.0	183	233	+2.5	+1.3	283
34	+6.0	+5.5	84	134	+0.9	+2.4	184	234	+0.5	+0.1	284
35	+5.2	+4.2	85	135	+1.4	+0.9	185	235	−1.2	+5.1	285
36	+1.6	+4.7	86	136	+6.1	+4.1	186	236	+4.1	+5.3	286
37	−2.4	+0.3	87	137	+2.8	+9.9	187	237	−2.4	+0.7	287
38	−5.7	+0.9	88	138	+8.4	+15.0	188	238	−2.3	−0.2	288
39	−0.9	+1.9	89	139	+4.7	+12.6	189	239	+0.9	+3.0	289
40	−0.2	−1.4	90	140	+8.8	+7.9	190	240	−1.3	+3.2	290
41	+0.5	−5.1	91	141	+5.3	+1.9	191	241	−2.7	−3.3	291
42	−0.8	−0.3	92	142	+1.9	+0.9	192	242	−1.4	+4.3	292
43	−0.7	−3.7	93	143	−4.5	+1.6	193	243	−3.2	−3.6	293
44	−4.0	−2.1	94	144	−5.1	−3.6	194	244	+0.4	−0.4	294
45	−0.4	−5.1	95	145	−3.0	−1.7	195	245	−2.9	−2.8	295

Mean=15.6 bushels.

Correlation coefficients were calculated for the yields of wheat in exactly the same manner as for oat hay. The data are presented in Table 6.

TABLE 6.—*Coefficients of correlation between yields of wheat grown on certain plots at Maggie, W. Va., in 1924*

Method of calculation	n	r
Harris's 2×2-fold contiguous	260	+0.565±0.028
Harris's 2×3-fold contiguous	258	+.516±0.031
Harris's 2×2-fold replicated	260	+.246±0.039
Ordinary "all contiguous plots"	260	+.553±0.030
Ordinary "paired contiguous plots"	130	+.617±0.037
Ordinary "paired replicated plots"	130	+.037±0.059

As in the case of the yields of oat hay, the coefficients of correlations based on the yields of wheat grain show that the experimental field was heterogeneous, and that the particular manner of plot replication was helpful in overcoming the objectionable effect of soil heterogeneity.

The correlation coefficients in Table 6 obtained by applying Harris's formula to the yields of contiguous plots are, for the 2×2-fold grouping, $+0.565\pm0.028$; and, for the 2×3-fold grouping, $+0.516\pm0.031$. The difference between the two coefficients is small and not significant. When the same formula is applied to replicated plots grouped in a 2×2-fold manner, the coefficient of correlation obtained is $+0.246\pm0.039$, or about one-half the magnitude of the coefficients based on contiguous plots. This coefficient is approximately six times its probable error and therefore statistically significant. However, the coefficient is low and shows that the correlation between the yields of replicated plots is not marked.

The correlation coefficient obtained by the ordinary method for "all contiguous plots" is $+0.553\pm0.030$, that for the "paired contiguous plots" is $+0.617\pm0.037$, and that for "paired replicated plots" $+0.037\pm0.059$. Here, as in the case of yields of oat hay, the correlation between the wheat yields of replicated plots is sensibly zero. The difference between the coefficients of yields of replicated plots based on the two methods of calculation is 0.209 ± 0.071, a difference slightly less than three times its probable error. In both the yields of oat hay and of wheat grain the correlation coefficients obtained by the ordinary method of calculation show a somewhat greater difference between contiguous and replicated plots than do the coefficients calculated by Harris's formula.

REGRESSION OF YIELDS IN CONTIGUOUS AND REPLICATED PLOTS

To obtain a graphic representation of the relationship between the yields in contiguous as compared with the yields in replicated plots, certain regression equations were calculated, and by means of these the regression lines were drawn. The data showing the correlation by the ordinary method of calculation between the yields of contiguous plots and between the yields of replicated plots for both the oat hay and wheat grain were used.

Before calculating the regression equations, appropriate tests were made to determine linearity. For this purpose the formula [6] suggested by Blakeman [7] for testing the significance of the difference between η^2 and r^2 was used. The correlation coefficients for "paired continguous plots" and "paired replicated plots" are given in Table 4 for the yields of oat hay and in Table 6 for the yields of wheat. The correlation ratios for the same sets of paired variables are given in Table 7.

[6] *P. E. diff.* $=2\times0.6745/\sqrt{n}\ \sqrt{\eta^2-r^2}\ \sqrt{(1-\eta^2)^2-(1-r^2)^2+1}$.

[7] BLAKEMAN, J. ON TESTS FOR LINEARITY OF REGRESSION IN FREQUENCY DISTRIBUTIONS. Biometrika 4: 332-350. 1905.

TABLE 7.—*Correlation ratios and the significance of the differences between the η^2 and r^2 values for the yields of "paired contiguous plots" and the yields of "paired replicated plots" at Maggie, W. Va.*

	Crop	n	$\eta^2 \pm$	Diff./η^2 & r^2/P.E.
"Paired contiguous plots"	Oat hay	130	0.729±0.028	1.9
"Paired replicated plots"	do	130	.373± .051	3.6
"Paired contiguous plots"	Wheat	130	.654± .034	1.9
"Paired replicated plots"	do	130	.206± .057	1.8

The correlation ratios based on the yields of oat hay are 0.729± 0.028 for the "paired contiguous plots" and 0.373±0.051 for the "paired replicated plots"; whereas the correlation ratios based on the yields of wheat grain are 0.654±0.034 for the "paired contiguous plots" and 0.206±0.057 for the "paired replicated plots." In all cases except one, the difference between η^2 and r^2 is not significant, as may be seen from the last column of Table 7. The exception is in the case of the "paired replicated plots" of oat hay. The question naturally arises whether the correlation ratio obtained here is of significance, or is due to accidental fluctuations. It is well known that relatively low values of η are quite likely to be too high, and consequently for paired variables which show little or no correlation the statistical value of η is questionable.

In Table 8 are the data from which the r and η values for the yields of oat hay in the "paired replicated plots" were calculated. This table shows several paired variables which are considerably removed from the main field of the distribution. It is possible that some of these outlying variables are accidental fluctuations and, as such, increase the magnitude of the correlation ratio.

TABLE 8.—*Correlation between yields of oat hay produced in 1923 on replicated plots of the rotation series at Maggie, W. Va.*

Deviation in pounds per acre of air-dry hay:	Deviation in pounds per acre of air-dry hay																	
	−850	−750	−650	−550	−450	−350	−250	−150	−50	+50	+150	+250	+350	+450	+550	+650	+750	
−550									1*									1
−450										1					1			2
−350						2	1	2	1			1	1	1				9
−250			1			1	4	1		1		3	1	1	1			14
−150		1	1		1	1	3	5	3	1	1	1						18
−50				1	1	2	3	3	3	3			1					17
+50				1		2	9	4	4	2				1				23
+150						1	4	4	2	2	1	2						16
+250					2		2	4		1			2					11
+350					1			3			1		1				1*	7
+450					1		1		1									3
+550									1									1
+650	1*						1											2
+750							1								1			2
+850							1	1	1									3
+950									1*									1
	1	1	2	2	6	9	30	27	18	11	3	7	6	3	3	0	1	130

$r = -0.068 \pm 0.059$.

In calculating the correlation ratio for the dependence of y on x in Table 8, it was found that a single paired variable contributed almost one-fourth of the total value obtained in the $N_x(\bar{y}_x-\bar{y})^2$ column. The value for $\Sigma N_x(\bar{y}_x-\bar{y})^2$ was 150.8398, whereas the value for $N_x(\bar{y}_x-\bar{y})^2$ of the first $\bar{y}$-array was 37.1161. Here is fairly good evidence that 1 of the 130 paired variables has contributed a disproportionate weight in determining the magnitude of the correlation ratio; and, moreover, it is quite possible that this outlying paired variable is accidental.

If the four outermost paired variables are eliminated, as is indicated by (e) in Table 8, the r and η values become -0.075 ± 0.060 and 0.331 ± 0.053, respectively. The difference between their squares is 0.104 ± 0.035, or 2.97 times its probable error. From this it follows

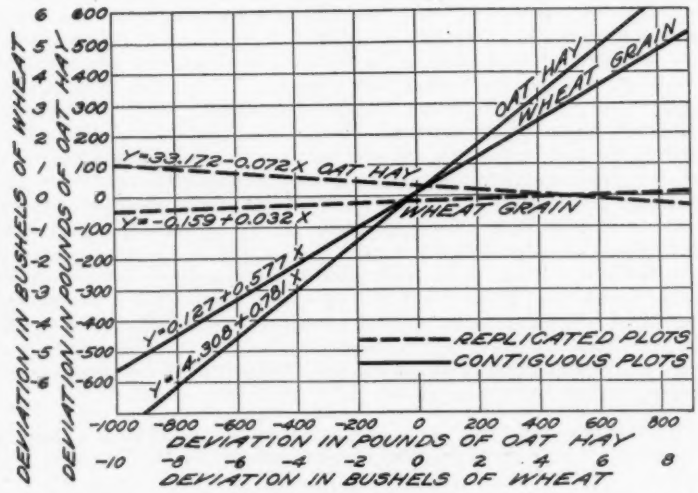

FIG. 2.—Regression lines for the yields of "paired contiguous plots" and of "paired replicated plots" in the rotation experiments at Maggie, W. Va.

that the odds are 1 to 22 that regression is linear. Although the odds in this case are rather high against the probability that regression is linear, it is certainly significant that by eliminating 4 of the 130 paired variables the odds are lowered from 65 to 1 to 22 to 1. In view of the above, a regression line for the yields of the "paired replicated plots" of oat hay was calculated and plotted, on the assumption that regression was linear.

The regression lines and their respective equations are shown in Figure 2. The effectiveness of replication in equalizing yield differences between plots due to soil heterogeneity is strikingly brought out. The regression lines for the yields of "paired replicated plots" are almost horizontal and show little or no correlation, whereas the regression lines for the yields of "paired contiguous plots" meet a horizontal at a marked angle and therefore show considerable correlation.

THE RELATION BETWEEN YIELDS OF OAT HAY AND WHEAT GRAIN

Harris[8] has pointed out that differences between the yielding capacity of plots in a particular experimental field may persist for a period of years. In other words, there is a correlation between the yields of plots in successive years. Table 9 was prepared in order to determine whether there was such a relationship between the yields of oat hay based on the net areas of the plots and wheat grain based on the rod-row samples. The correlation coefficient obtained ($+0.364\pm0.036$) indicates that there is a significant correlation between the yield of oat hay in 1923 and the yield of wheat grain in 1924. Apparently there was less correlation between the yields of oat hay and wheat grain on the same plots in the two successive years the experiment was carried on than there was between yields of contiguous plots in either of the two years. This undoubtedly was due to seasonal influence and the fact that one-year yields were expressed in terms of oat hay and the other year in terms of wheat grain.

TABLE 9.—*Correlation of yield of wheat grain in 1924 with yield of oat hay in 1923 at Maggie, W. Va.*

	Oat hay, pounds per acre																		
	1,100	1,300	1,500	1,700	1,900	2,100	2,300	2,500	2,700	2,900	3,100	3,300	3,500	3,700	3,900	4,100	4,300	4,500	
Wheat grain, bushels per acre:																			
7.5				1			1												2
9				4	2	1													7
10.5	1	2	1	7	6	4	3												24
12		2	5	8	10	6	3												34
13.5		1	5	14	4	2	2		3										31
15	1		6	17	15	7	3	1	1										51
16.5			5	18	11	7	1			1									43
18			3	11	14	5	1												34
19.5			2	5	6	2													15
21			2	6	2	3	1	1		1			1						17
22.5									1										1
24					1		2												3
25.5						1		1											2
27								1	1										2
28.5								1											1
30						1												2	3
	2	5	29	91	71	39	17	5	6	2	0	0	1	0	0	0	0	2	270

[1] $=0.364\pm0.036$.

Certain other correlation coefficients showing the relationship between yields of oat hay and wheat were also calculated. The coefficients obtained for the yield of oat hay and each of the following wheat yields—calculated grain on the entire plot, grain plus straw based on rod-row samples, and grain plus straw determined from the entire plot—were $+0.035\pm0.035$, $+0.385\pm0.035$, and $+0.394\pm0.035$, respectively. The magnitudes of these coefficients of correlation, and of the one mentioned in the preceeding paragraph as well, are practically of the same order and do not differ significantly from one another.

[8] HARRIS, J. A., and SCOFIELD, C. S. PERMANENCE OF DIFFERENCES IN THE PLOTS OF AN EXPERIMENTAL FIELD. Jour. Agr. Research 20: 335–356. 1920.

SUMMARY AND CONCLUSIONS

An experimental field at Maggie, W. Va., containing 270 plots, each with a gross area (68×21 feet) of approximately $\frac{2}{61}$ of an acre, was studied to determine the degree of soil heterogeneity that existed. As a basis for this study the yield of oat hay in 1923 and wheat grain in 1924 was used.

The yield of air-dry oat hay was determined by weighing the production of the entire net area (61×14 feet, or $\frac{1}{51}$ of an acre) of each plot. The yield of wheat in bushels per acre, and the weight of the straw plus grain, were determined from five rod rows removed from certain places in each plot. The air-dry weight of the total grain plus straw produced on the net areas of each plot was also determined.

The five rod rows of wheat showed a high correlation ($+0.951\pm0.004$) between the weight of the grain and the weight of straw plus grain. A high correlation ($+0.841\pm0.012$) was also found between the weight of the grain plus straw on the entire net area of each plot and the weight of the grain produced by rod-row sample removed from each plot. It was considered that yield based on a sample of five rod road removed from a plot in the manner described constituted a fairly trustworthy index as to the yield of that plot.

A marked correlation was found between the yields of oat hay in contiguous plots, both when calculated in the usual way and when calculated by the formula suggested by Harris. The coefficients of correlation ranged from $+0.542\pm0.030$ to $+0.694\pm0.031$. The coefficients of correlation between yields of replicated plots were $+0.134\pm0.041$ and -0.068 ± 0.059.

As in the case of oat hay, the wheat grain showed a marked correlation between the yields of contiguous plots ($r=+0.516\pm0.031$ to $r=+0.617\pm0.037$) and slight correlation between the yields of replicated plots ($r=+0.246\pm0.039$ and $r=+0.037\pm0.059$).

In the case of oat hay and of wheat grain there was somewhat less difference between the coefficients of correlation for contiguous plots and replicated plots when calculated by Harris's formula than when calculated in the ordinary way. The correlation coefficients for the yields of replicated plots by the ordinary method of calculation were sensibly zero.

It is concluded that the particular experimental field under observation is not naturally uniform with respect to productivity. Plot replication is effective in equalizing yield differences due to soil heterogeneity, and thus helps to overcome vitiating "place effect" in field experiments such as the one contemplated here.

There was some tendency for the plots which produced relatively high yields of oat hay in 1923 to produce relatively high yields of wheat grain in 1924. The correlation coefficient between the plot yields of these two successive years was $+0.364\pm0.036$.

A FORMULA FOR ESTIMATING SURFACE AREA OF DAIRY CATTLE [1]

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INTRODUCTION

It has long been accepted as a fact that heat production in the animal body is due to the oxidation processes within the body, and hence is an accurate measure of the rate of metabolism. In order to utilize this standard in research, it becomes necessary to devise a satisfactory method for comparing the heat production of animals of different sizes.

Very early in nutritional investigations it became apparent that that the heat production of an animal as not proportional to its live weight. In 1848 Bergmann (*3*) [3] [4] attempted to explain the relatively higher heat production of smaller animals per unit of weight by the generalization that the heat production of the animal body is proportional to its surface area. This idea found wide application and was given strong support by many investigators.

Regnault and Reiset (*10, p. 514*) [5] in their studies of the respiratory exchange of different species under diverse conditions, determined that the oxygen consumption of animals is not proportional to their weight. Obviously the heat production would have a similar ratio per unit of weight, which they explained as being due to the fact that small animals expose a relatively greater surface area to the cooling effect of the atmosphere and consequently require a greater heat production to maintain their body temperature. Some years later Rubner [6] proved that their explanation was faulty, although their general statement of facts was correct.

Thus far the idea of the relation between heat production and surface area had been entirely theoretical, since no actual measurements of the surface area of living subjects were available. In 1879 Meeh (*8*) published the actual measurements of the surface area of living subjects, the results of his own painstaking work, which included the measurements of 6 adults and 10 children.

From his measurements Meeh developed a formula by which the surface area of any individual could be determined. This formula

[1] Received for publication Dec. 26, 1925; issued August, 1926.

[2] The writer wishes to express his appreciation to A. C. Ragsdale, Professor of Dairy Husbandry, and to Samuel Brody, Assistant Professor of Dairy Chemistry, University of Missouri, for aid in outlining the problem and for helpful suggestions in interpreting and summarizing data and in the preparation of the manuscript. The major credit for developing the idea and details of the revolving metal cylinder of the measuring instrument, is due Samuel Brody. In this he was assisted by the writer. A. C. Ragsdale, C. W. W. Turner, and Chester Sparrow (an engineering student) all had a part in developing the plan and in perfecting this new method of measuring the surface area of animals. All experimental data were taken by the author and have been presented by him in a thesis submitted at the University of Missouri in partial fulfillment of the requirements for the degree of master of arts.

[3] Reference is made by number (italic) to "Literature cited", p. 278.

[4] Cited by Benedict (*1*).

[5] Cited by Lusk (*7, p. 120.*)

[6] Cited by Lusk (*7, p. 121*).

was based on the fundamental mathematical law that the surface areas of similar solids are proportional to the two-thirds power of their volumes.

Assuming that the specific gravity was the same in each case, he substituted weight for volume so that his formula is expressed in the following form:

$$S = K W^{\frac{2}{3}}$$

in which S is the surface area in square centimeters, W the weight in kilograms, and K a constant (12.312 for adults and 11.9 for children).

In 1883 Rubner (*11*) measured the surface area of a number of dogs varying greatly in weight, and also determined their heat production under comparable conditions. He found the body heat production to be quite constant per square meter of the body surface, but varying greatly per kilogram of body weight. A few years later, observations by Voit (*13*) indicated that this law is applicable over a wide range in the animal kingdom. He determined the heat production of subjects varying in size from a mature horse to a mouse and found the heat production per meter of body surface to be quite constant in all cases.

In 1916 D. Du Bois and E. F. Du Bois (*4*) measured the surface area of a number of human subjects, and from their measurements they developed a formula for estimating the surface area of humans based on the weight and height of the individual.

In general, investigators both in human physiology and pathology and in animal production have accepted the practice of calculating heat production per unit of surface area, and consequently surface area is the most common unit of reference in estimating basal metabolism.

Benedict (*5, p. 129*) and his coworkers have challenged this practice of calculating heat production per unit of body surface. They maintain that the heat production depends upon the actual mass of protoplasmic tissue within the body and not upon the cooling on the body surface. They have published an extensive series of prediction tables for determining heat production based on weight, height, sex, and age, but involving no assumption concerning derivation of surface area.

Extensive comparisons of the Benedict standard and the body-surface standard of Du Bois, show that the results obtained are almost parallel. More recently Benedict (*2, p. 159*) stated: "We believe that the accurate measurements of body-surface made possible by Du Bois may legitimately be used in a manner heretofore never practicable in metabolism experiments, provided that they are considered as physical measurements and with no erroneous conceptions as to the existence of a causal relationship between surface area and heat production."

Assuming that the surface area of the body is an accurate standard for estimating the metabolism, the investigator meets with difficulty because it has been impossible to calculate the surface area of animals with any degree of accuracy.

Earlier investigators have accepted the Meeh formula as applying to all types of animals, but the correct constants have been worked out in only a few instances, and also later investigations proved that in many instances this formula gave very erroneous results.

Trowbridge, Moulton, and Haigh (*12*) published a number of measurements of the surface area of cattle and calculated the constant for the Meeh formula. The constants varied from 7.319 to 10.74, depending on the age and the degree of fatness of the animals.

In 1916 Moulton (*9*) developed two formulas for estimating surface area of cattle, based on warm empty weight, but using a different exponent than the one used by Meeh. His formula for fat cattle is:

$$A = 0.158 W^{\frac{2}{3}}$$

and for other animals:

$$A = 0.1186 W^{\frac{2}{3}}$$

in which A is the surface area in square meters, and W is the empty weight in kilograms.

Hogan and Skouby (*6*) considered the work of Trowbridge, Moulton, and Haigh, and that of Moulton, as proof that the surface area of cattle could not be accurately calculated as a power function of weight, and they developed a formula for estimating surface area of cattle and swine, patterned after the height-weight formula of Du Bois (*4*), in which both weight and body length are factors. Their formula is:

$$S = W.^{4} \times L.^{6} \times K$$

in which S is surface area in square centimeters, W the live weight in kilograms, L the length of the body in centimeters, and K a constant (217 for cattle and 175 for swine).

The purpose of the work set forth in this paper was to actually measure the surface area of normal dairy cows, and to develop a simple formula for estimating the surface area of dairy cattle.

MEASUREMENT OF SURFACE AREA

It was rather difficult to find a satisfactory method for measuring the surface area of living animals with a fair degree of accuracy. The method finally used consisted in rolling a revolving metal cylinder of known area, attached to a revolution counter, over the entire surface of the animal, and then simply multiplying the number of revolutions of the roller by the area of the roller. This gave an approximately accurate surface-area measurement with a very small amount of computation, and the measuring instrument was not difficult to use.

The technique involved in taking surface area was simple. In most cases, only half of the body surface the right side of the animal was actually measured, and the results were multiplied by two. First, the dorsal and ventral median lines were marked with colored crayon. Then, starting at the base of the horn, the roller was passed along the dorsal median line to the posterior mid line. Lower down on the side of the animal, measurement was from the outline of the jawbone to the posterior mid line. The roller was equipped with a crayon marker which plainly marked the path of the roller. Then, by keeping the outer edge of the roller along the mark made the previous trip, the entire surface was covered. The only region which was difficult to measure accurately was around the udder and inside of the hind leg, but with a little extra care this was accomplished very satisfactorily. The legs were measured by moving the roller spirally

down them. The head and ears were measured last. The area of the tail was not measured with the roller, but it was estimated by multiplying the length, a figure representing the average of the diameters at the root and at the base of the switch.

Figures 1 and 2 are illustrations of the measuring apparatus. *A* is the brass roller. This roller is 2 inches long and 2 inches in diameter. Repeated trials with rollers of different lengths and diameters established this one as most satisfactory. A smaller roller did not turn uniformly, and a larger one was inconvenient in measuring around the flank, udder, armpit, etc. *B* is the milled rim of a disk which served as a marker. *C* is the dial of the revolution counter. *D* is the handle. *E* is a metal tube containing a fine spring which holds the crayon against the milled rim of the marking

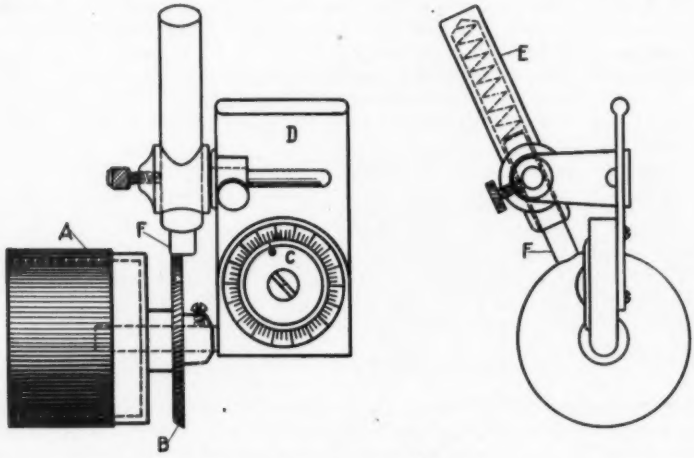

FIG. 1.—Instrument used for measuring surface area of animal. *A*, brass cylinder; *B*, milled rim of disk which makes a chalk line; *C*, revolution counter; *D*, handle; *E*, metal tube containing spring; *F*, chalk crayon. (Drawing from photograph)

disk. F is the crayon. The milled rim made a fine but distinct chalk line on the animal. Figure 3 is a photograph of the measuring operation.

An effort was made to determine the quantitative error in the measured area as compared to the actual surface.

Two animals—cow 129 and heifer 27—were slaughtered. Both animals were measured with the roller on the morning before they were slaughtered. After they were slaughtered, the outlines of the hides were traced on paper and the areas of the tracings were measured. The results obtained are as follows:

Cow 129

Body area of live cow	42,635 sq. cm.
Area of hide	42,883 sq. cm.
Difference	+248 sq. cm.
Per cent difference (based on area of hide)	+0.58 per cent

Heifer 27

Body area of live heifer	14,821 sq. cm.
Area of hide	14,701 sq. cm.
Difference	−120 sq. cm.
Per cent difference (based on area of hide)	−0.82 per cent

The two methods gave approximately the same results.

DESCRIPTION OF ANIMALS USED

The data presented in this paper were all taken on Holstein and Jersey animals. Practically every animal of these two breeds in the University of Missouri dairy herd have been measured, and in some cases, in order to obtain more data, certain young animals were measured two or more times at different ages. The data include

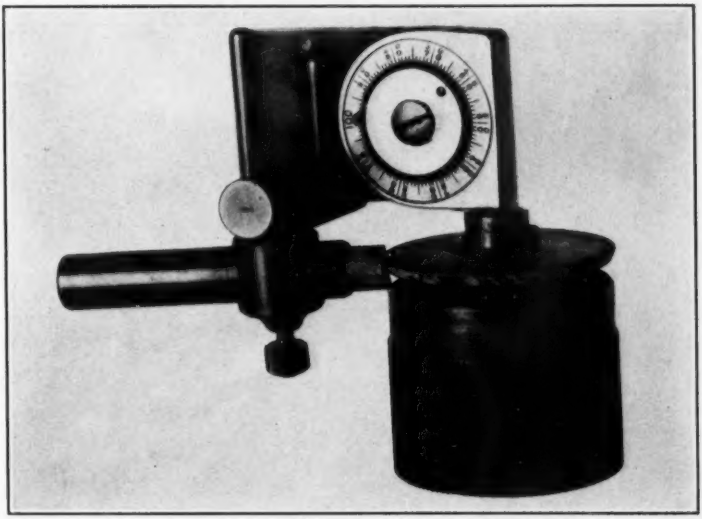

FIG. 2.—Photograph of instrument for measuring surface area of animal

measurements of animals varying in age from a few hours to 10 years, and in weight from 17.6 to 653 kilograms. The animals represent many variations in degree of fleshing, body conformation, and stages of lactation and gestation.

LIVE WEIGHT OF ANIMALS

In all cases the weight of the animal was taken immediately before the surface area was measured. No attempt was made to withhold either feed or water before weighing.

DEVELOPMENT OF FORMULA

When the live weight was plotted against surface area, as in Figure 4, a simple parabolic curve was obtained with all the points falling reasonably close to the curve. Such a curve indicated that surface area is a direct power function of weight, and hence may be determined by a simple formula as

$$X = Ky^n$$

By plotting surface area against weight on logarithmic paper as in Figure 4, a straight line was obtained having a slope of 0.56 which designated the value of the exponant n in the equation. In general, the values for the Holsteins were slightly greater than those for Jerseys (fig. 4). In reality, the value 0.57 for Holsteins and of 0.55 for Jerseys is more nearly exact than the average figure 0.56, but a few trial calculations indicated that the slight increase in accuracy of results obtained by using a different exponent in the formula for the different breeds was not sufficient to justify the use of two separate formulae.

Knowing the live weight and the surface area of the animals and the value of n, it remained to solve for K in the formula. By choosing values that fell on the weight-surface area curve, and substituting them in the formula, it was possible to solve for K.

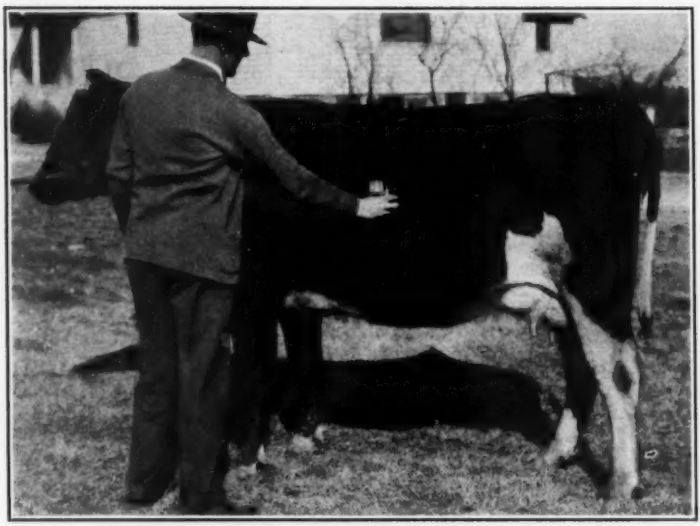

FIG. 3.—Measuring the surface area of a cow, with the instrument shown in Figures 1 and 2

$$S\ A = K W^n \text{ or } \frac{S\ A}{W^n} = K$$

Using logarithms, the equation becomes

$$\text{Log } S\ A - 0.56 \text{ Log } W = \text{Log } K$$

The value of K was 1,470. Then the formula in its final form becomes

$$S\ A = 1{,}470\ W^{0.56}$$

in which $S\ A$ is the surface area in square centimeters, W is the live weight in kilograms, and 1,470 is a constant.

The values of W for the different animals were substituted in the formula, and the equations solved to determine the surface area. These computed values for surface area were then compared with the observed values. These results are given in Table I. In all but 4

cases, or in 96 per cent of all animals measured, the computed value was within ±5 per cent of the observed value. The remaining 4 animals had computed values varying from 5.4 per cent to 6.17 per cent above the observed values. The average per cent deviation in all animals, regardless of sign, was 1.95. Among the 46 animals having a

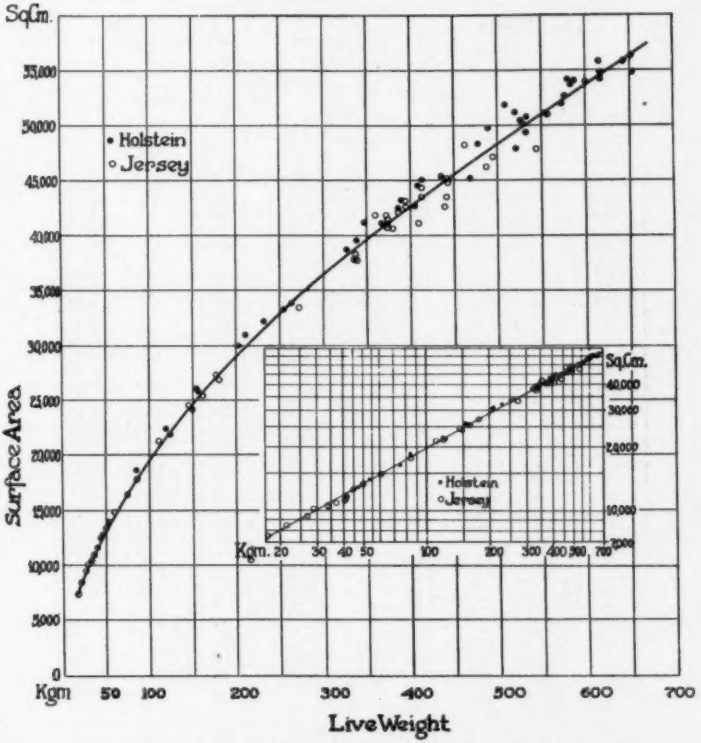

FIG. 4.—Relation between the live weight and the surface area of dairy cows

computed value greater than the observed value the average per cent of deviation was 1.99, and among the 50 animals having a computed value lower than the observed value the average per cent of deviation was 1.92.

The area was also computed according to the Meeh formula (8). These results are given in Table I in order that they may be compared with the values obtained from the new formula.

By plotting surface area against weight on logarithmic paper as in Figure 4, a straight line was obtained having a slope of 0.56 which designated the value of the exponant n in the equation. In general, the values for the Holsteins were slightly greater than those for Jerseys (fig. 4). In reality, the value 0.57 for Holsteins and of 0.55 for Jerseys is more nearly exact than the average figure 0.56, but a few trial calculations indicated that the slight increase in accuracy of results obtained by using a different exponent in the formula for the different breeds was not sufficient to justify the use of two separate formulae.

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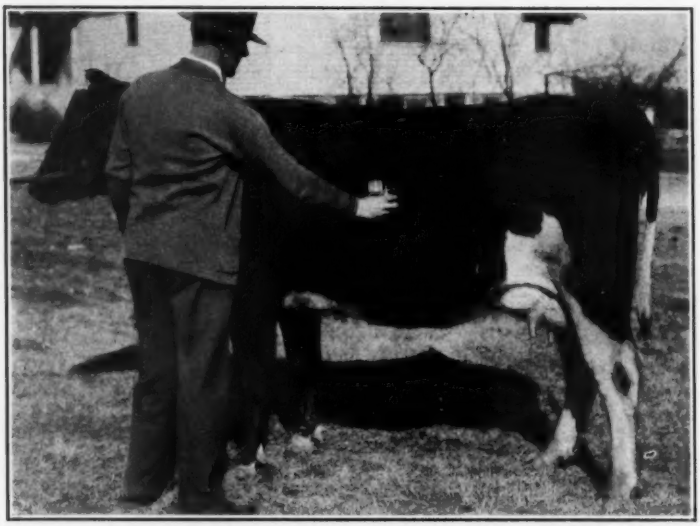

FIG. 3.—Measuring the surface area of a cow, with the instrument shown in Figures 1 and 2

$$S\,A = K\,W^n \text{ or } \frac{S\,A}{W^n} = K$$

Using logarithms, the equation becomes

$$\text{Log } S\,A - 0.56 \text{ Log } W = \text{Log } K$$

The value of K was 1,470. Then the formula in its final form becomes

$$S\,A = 1{,}470\ W^{0.56}$$

in which $S\,A$ is the surface area in square centimeters, W is the live weight in kilograms, and 1,470 is a constant.

The values of W for the different animals were substituted in the formula, and the equations solved to determine the surface area. These computed values for surface area were then compared with the observed values. These results are given in Table I. In all but 4

cases, or in 96 per cent of all animals measured, the computed value was within ±5 per cent of the observed value. The remaining 4 animals had computed values varying from 5.4 per cent to 6.17 per cent above the observed values. The average per cent deviation in all animals, regardless of sign, was 1.95. Among the 46 animals having a

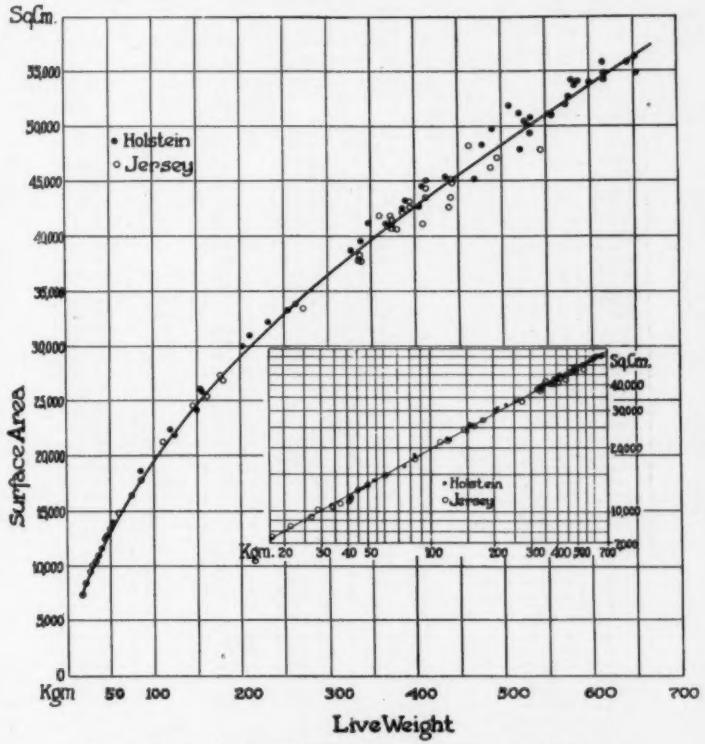

FIG. 4.—Relation between the live weight and the surface area of dairy cows

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The area was also computed according to the Meeh formula (8). These results are given in Table I in order that they may be compared with the values obtained from the new formula.

TABLE 1.—*Data used in developing formula and computed values*

JERSEYS

Herd No. of animal[a]	Age of animal	Live weight, kgm.	Surface area				
			Measured	Computed			
				$S=1470\ W^{0.58}$		$S=839\ W^{2/3}$	
			Surface, sq. cm.	Surface, sq. cm.	Error, per cent	Surface, sq. cm.	Error, per cent
1	10 hours	17.6	7,296	7,375	1.08	5,676	−22.20
2	2 days	21.5	8,433	8,232	−2.38	6,466	−23.33
170	3 days	27.0	9,404	9,421	0.18	7,587	−19.32
187	16 days	29.0	10,076	9,767	−3.06	7,918	−21.41
189[1]	23 days	34.0	10,557	10,681	1.17	8,803	−16.61
188[1]	35 days	34.0	10,395	10,681	2.75	8,803	−15.31
189[2]	38 days	40.5	11,368	11,785	3.66	9,893	−12.97
188[2]	40 days	41.5	11,692	11,980	2.46	10,088	−13.71
186[1]	44 days	41.0	11,469	11,867	3.47	9,974	−13.03
157[1]	50 days	34.0	10,443	10,681	2.27	8,803	−15.70
157[2]	70 days	37.0	10,954	11,201	2.25	9,314	−14.97
164	80 days	50.0	13,466	13,267	−1.47	11,381	−15.48
185[1]	72 days	45.0	12,671	12,505	−1.31	10,613	−16.24
27	3 months	61.0	14,821	14,839	0.12	12,997	−12.30
182[1]	4 months	85.0	17,892	17,883	−0.05	16,223	−9.32
181[1]	5 months	111.0	21,291	20,779	−2.40	19,373	−9.00
186[2]	6 months	122.0	21,672	21,915	1.12	20,633	−4.79
183	6 months	161.0	25,510	25,614	0.41	24,823	−2.69
185[2]	6 months	145.0	24,540	24,160	−1.54	23,150	−5.66
182[2]	7 months	177.0	27,294	27,016	−1.02	26,359	−3.42
182[2]	8 months	179	26,817	27,188	1.38	26,640	−0.66
181[2]	9 months	181	26,970	27,358	1.44	26,753	−0.80
180	12 months	272	33,423	34,401	2.92	35,209	5.34
180	1 year, 4 months	336	37,853	38,744	2.35	40,395	6.71
177	1 year, 7 months	324	38,964	37,959	−2.57	38,664	−0.77
176	1 year, 7 months	336	38,365	38,744	0.98	40,537	5.66
173	1 year, 10 months	374	41,051	41,151	0.24	43,536	6.05
172	1 year, 11 months	395	43,094	42,435	−1.53	45,151	4.77
171	1 year, 11 months	359	41,959	40,215	−4.15	42,364	0.96
170	2 years, 1 month	413	43,544	43,512	−0.07	45,512	4.51
166	2 years, 7 months	374	40,750	41,151	0.98	43,536	6.83
167	2 years, 7 months	372	41,822	41,027	−1.90	43,382	3.73
165	2 years, 8 months	381	40,609	41,583	2.39	44,078	8.54
164	2 years, 11 months	338	37,773	38,874	2.91	40,696	7.73
157	4 years, 1 month	374	41,424	41,151	−0.65	43,536	5.10
156	4 years, 1 month	395	42,595	42,435	−0.37	45,151	6.00
154	4 years, 10 months	463	48,263	46,401	−3.85	50,575	4.79
151	5 years, 3 months	545	47,900	50,858	6.17	55,958	16.82
129	7 years	442	42,635	45,205	6.02	48,684	14.18
126	7 years, 4 months	495	47,161	48,179	2.15	52,482	11.28
127	7 years, 7 months	444	44,850	45,320	1.02	48,811	8.83
120	7 years, 10 months	386	42,135	41,889	−0.58	44,463	5.52
121	8 years	442	43,430	45,205	4.08	48,665	12.05
110	8 years, 1 month	487	46,189	47,739	3.35	51,913	12.39
125	8 years, 9 months	442	45,106	45,205	0.21	48,666	7.89
108	9 years	410	41,112	43,334	5.40	46,287	12.58

[a] Superior numbers indicate animals measured more than one time.

TABLE 1.—*Data used in developing formula and computed values*—Continued

HOLSTEINS

Herd No. of animal	Age of animal	Live weight, kgm.	Surface area				
			Measured	Computed			
				$S=147O\ W^{0.56}$		$S=839\ W^{2/3}$	
			Surface, sq. cm.	Surface, sq. cm.	Error, per cent	Surface, sq. cm.	Error, per cent
552	1 day	47.5	12,847	12,907	0.46	11,017	−14.24
274	3 days	53.5	13,982	13,782	−1.43	11,910	−14.81
554	7 days	41.0	11,600	11,867	2.30	9,974	−14.01
553	9 days	44.5	12,573	12,411	−1.28	10,517	−16.35
550	7 weeks	75.0	16,466	16,667	1.22	14,922	−9.37
551	3 months	84.0	18,660	17,764	−4.80	16,087	−13.78
549	3½ months	120.0	22,376	21,611	−3.42	20,406	−8.80
548	4 months	125.0	21,817	22,210	1.80	20,847	−4.45
545	4½ months	150.0	24,061	24,615	2.30	23,668	−1.63
546	5 months	154.0	26,159	24,981	−4.50	24,105	−7.85
547	6 months	156.0	25,820	25,209	−2.36	24,306	−5.86
544[1]	7 months	202.0	30,081	29,235	−2.81	28,875	−4.00
544[2]	7½ months	209.0	31,041	29,663	−4.43	29,444	−5.14
542	8 months	231.0	32,260	31,381	−2.72	32,709	1.39
541	10 months	254.0	33,335	33,102	−0.69	33,528	0.58
540	1 year	263.0	33,870	33,757	−0.33	34,315	1.31
537[1]	1 year, 2 months	336.0	39,591	38,744	−2.14	40,646	2.66
534	1 year, 4 months	327.0	38,680	38,157	−1.35	39,824	2.95
535	1 year, 4 months	408.0	44,499	43,216	−2.88	46,136	3.67
537[2]	1 year, 5 months	389.0	43,194	42,062	−2.62	44,533	3.09
536	1 year, 6 months	469.0	45,242	46,728	3.28	50,626	11.90
533	1 year, 6 months	413.0	45,091	43,512	−3.50	46,515	3.15
538	1 year, 6 months	406	42,604	43,096	1.15	45,986	7.93
532	1 year, 7 months	368	41,076	40,778	−0.72	43,086	4.89
531	1 year, 8 months	347	41,200	39,446	−4.25	41,432	0.56
530	1 year, 8 months	386	42,495	41,889	−1.42	44,481	4.67
527	1 year, 10 months	436	45,384	44,859	−1.15	48,253	6.32
526	2 years, 2 months	533	50,841	50,224	−1.21	55,133	8.44
525	2 years, 5 months	553	51,165	51,276	0.22	56,503	10.43
521	2 years, 11 months	644	55,925	55,864	−0.11	62,570	11.88
518	3 years, 1 month	531	50,138	50,119	−0.04	55,017	9.73
517	3 years, 9 months	601	54,044	53,733	−0.58	59,752	10.56
515	4 years	616	55,937	54,185	−3.13	60,345	7.88
512	4 years, 2 months	508	51,914	48,885	−5.83	53,395	2.85
510	4 years, 3 months	576	52,737	52,465	−0.52	58,083	10.14
509	4 years, 3 months	490	49,805	47,904	−3.81	52,149	4.70
508	4 years, 5 months	478	48,319	47,241	−2.23	51,293	6.15
507	4 years, 6 months	574	52,012	52,362	0.67	57,950	11.41
503	5 years	617	54,334	54,535	0.37	60,809	11.91
288	6 years	579	54,240	52,620	−2.98	58,261	7.41
290	6 years	653	54,818	56,302	2.71	63,151	15.20
292	6 years	592	54,172	53,281	−1.64	59,154	9.19
285	6 years, 2 months	522	47,883	49,640	3.66	53,158	11.01
281	6 years, 7 months	526	50,521	49,852	−1.32	54,672	8.21
281	6 years, 8 months	556	51,008	51,432	0.83	56,731	11.21
279	7 years, 4 months	576	52,805	52,466	−0.64	58,083	10.00
274	7 years, 5 months	608	53,710	54,085	0.70	60,214	12.11
275	7 years, 5 months	520	51,278	49,532	−3.40	54,254	5.80
266	8 years, 5 months	535	49,399	50,331	1.88	55,293	11.93
254	10 years	617	54,830	54,534	−0.53	60,810	10.90

DISCUSSION

A comparison of the computed values by the two formulae shows that a much greater range of error was obtained by using the two-thirds power of weight than by using the 0.56 power. The former gave results which were much too low in very small animals, and much too high in large animals, the percentage of error ranging from −23.2 per cent in 2-day-old calf to +16.8 per cent in a very fat barren cow. It was found that the constant 839 gave the most uniform results, but even then the range of error was so great that such results are of little value.

It is hardly fair to compare results obtained by the new formula with those obtained by Moulton's formula (*9*), inasmuch as his formula is based on warm empty weight rather than live weight. A few trial computations, substituting live weight for W in Moulton's formula, showed that when the five-eighths power of weight is used the results again are too low in very small animals and too great in large animals, especially in those which are rather fat.

The results obtained by using the 0.56 power of weight were in close agreement with the observed values. Of the four animals whose computed areas varied more than 5 per cent from the observed areas three were abnormal individuals. Cows 129 and 151 were nonbreeders. Both were dry and very fat when measured. Cow 108 was a very compactly built, short-legged individual, which was dry and quite fat when measured.

The possibility of introducing a second variable factor into the formula was discarded, since it was found possible to determine surface area quite accurately as a simple power function of weight. A second variable would only complicate the formula without increasing its accuracy, because the probable error in the observed value makes it questionable whether results closer than ±5 per cent are possible.

SUMMARY AND CONCLUSIONS

In normal dairy cattle, surface area has been found to be a direct power function of weight. The formula $SA = 1470\ W^{0.56}$, in which SA is the surface area in square centimeters, and W the live weight of the animal in kilograms, accurately expressed the relationship between surface area and live weight.

Both the two-thirds and the five-eighths powers of the weight gave results which were much too low in very small individuals, and much too high in large or extremely fat animals.

The surface areas of 96 dairy cattle were measured. The areas of 92 animals as computed by the proposed formula were within ±5 per cent of the observed areas, and the maximum error in the case of an extremely fat barren cow was only 6.2 per cent. The introduction of linear measurements into the formula would only complicate it without increasing its accuracy.

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THE CAUSE AND CONTROL OF YELLOW BERRY IN TURKEY WHEAT GROWN UNDER DRY-FARMING CONDITIONS.[1]

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INTRODUCTION

Under a cooperative agreement between the Bureau of Plant Industry of the United States Department of Agriculture and the Oregon Agricultural Experiment Station, a farm of 230 acres at Moro, Oreg., has been operated as a dry-land experiment station since 1911. Located in a region in which the rainfall is between 12 and 13 inches, its sole purpose is to solve problems that are peculiar to the dry-farmed areas of the Central Columbia River Basin.

The typical dry-farmed lands in this region are rich in the mineral elements of plant food. In composition, their weakness lies in their limited amount of native organic matter, which in time must fail to yield, in ordinary processes of decomposition, the requisite amount of available nitrogen to meet fully the crop requirements in the matter of growth. Since climate and rainfall practically limit the cropping of the dry-farmed lands to the small grains, no satisfactory means are apparent as yet for guarding against ultimate depletion of native organic matter and nitrogen.

TILLAGE EXPERIMENTS

Early in the development of experiment station work at Moro, a series of tillage plots was laid out for the purpose of determining what might be the effect on the accumulation of soil moisture and grain yields of variation in the tillage practice of the summer fallow. The plan of the work will be understood readily from the arrangement of the plots shown in Figure 1, if while studying that arrangement it is noted that each year while three tiers of plots of early spring, medium-early spring, and late-spring plowing were being summer-fallowed, three other adjacent tiers, identical plot for plot in previous summer-fallowed treatment, were in crop. All kinds of summer-fallow tillage were to be practiced, including extremes of thoroughly good and thoroughly poor. Turkey wheat was the crop to be grown.

EARLIER FIELD OBSERVATIONS

Although this series of plots was planned originally for the study of the effect on yields of various methods of summer-fallow treatment, it was noted as long ago as 1914 (*7*, *8*) that grain from the differently treated plots varied strikingly in its percentage of yellow berry. Plots given good summer-fallow treatment produced not only the largest amount of grain and straw, but always grain with the least percentage of yellow berry.

[1] Received for publication Nov. 27, 1925; issued August, 1926.

Normally, the kernels of Turkey wheat are hard and flinty. Cross sections of them are translucent in appearance, there being no visible indication of starch deposits. Yellow-berry kernels are lacking in hardness, and the clear, dark red color that is characteristic of Turkey wheat at its best. The mottled appearance is evidence of deteriorated quality. Upon cross-sectioning, starchy deposits are clearly evident. If mottling is very pronounced offerings may be docked several cents per bushel under prevailing prices for the best Turkey. Turkey wheat is the variety grown most in this particular region. It stands high in the estimation of producers and millers. Since yellow berry to the extent of 50 per cent or more is of frequent occurrence, it appeared from these earlier field observations on the

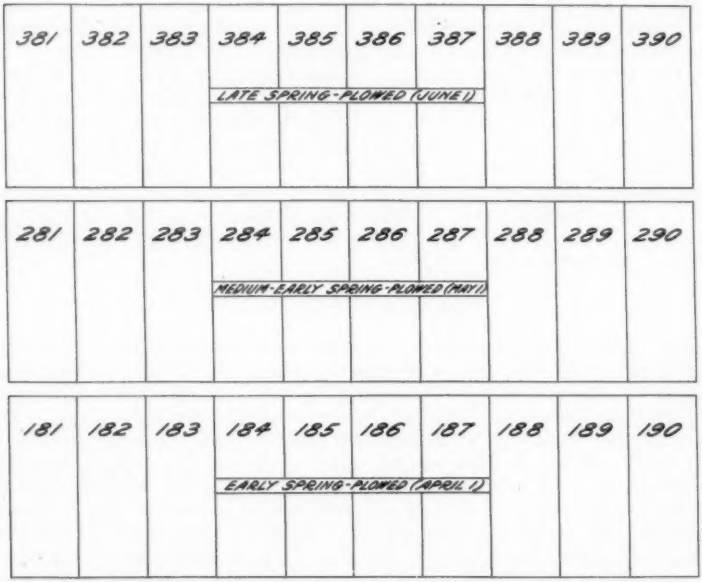

FIG. 1.—Arrangement of tillage plots. This series alternates in fallow and crop with a similarly arranged series, numbered 481–490, 581–590, and 681–690

Nos. 181, 281, and 381, packed with surface packer, and frequently cultivated. Clean summer fallow.
Nos. 182, 282, and 382, no cultivations of summer fallow after plowing. Weeds hoed off before maturity. Neglected fallow.
Nos. 183, 188, 283, 288, 383, and 388, check plots, spring-disked before plowing. Clean fallow
Nos 184, 284, and 384, one harrowing after plowing. No further cultivation. Weeds hoed off before maturity. Neglected fallow.
Nos. 190, 290, and 390, fall-disked and spring-disked before plowing. Frequent cultivation. Clean fallow.
(Size of plots: 2 rods by 8 rods. Width of alleys between plots, 4 feet 7 inches. Width of roadway between tiers, 1 rod.)

tillage plots that advantage might be taken of them—and without change in the original plans for them—to try to find the fundamental cause of yellow berry and means for lessening its prevalence.

GOOD AND POOR SUMMER FALLOW

"Good" summer-fallow treatment implies fall or early spring disking before plowing, reasonably early spring plowing and cultivation with harrow and weeder sufficient to keep the surface soil loose and the plot clean of weeds. It is just such treatment as the

thoroughgoing grower will give his summer fallow when determined to retain in it the greatest percentage of soil moisture possible. "Poor" summer-fallow treatment means no disking previous to plowing, late spring plowing, and little if any summer cultivation. It is the treatment given his summer fallow by one who, for one reason or another, fails to employ the best methods for conserving soil moisture.

Laboratory data on the crops of 1918 and 1919 confirmed an estimate one might have made readily from field observations as to the relative values for milling purposes of the grains from the various tillage plots. Flour millers place a premium on high content of protein in wheat, and in this respect the grain from the well-tilled plots had a decided advantage. These earlier field and laboratory observations are incorporated along with subsequent observations of like kind in Table 1.

TABLE 1.—*Field and laboratory data for crops grown on well-tilled and poorly-tilled summer fallow, 1918 to 1924, inclusive*

Year	Tillage treatment and average number of plots	Yields in pounds			Ratio of grain to straw	Physical characteristics of grain				Protein on dry basis	
		Total	Grain	Straw		Weight		Flinty, per cent by weight	Yellow berry, per cent by weight	In grain	In straw
						Per bushel	Per 1,000 kernels				
						Lbs.	*Gm.*			*P. ct.*	*P. ct.*
1918	{Good, 7	4,714	1,241	3,473	1:2.8	57.6	20.3	98.6	1.4	17.32	.....
	{Poor, 7	3,744	1,281	2,463	1:1.9	60.0	24.6	61.7	38.3	11.69	.....
1919	{Good, 4	7,180	2,148	5,032	1:2.3	60.0	22.1	96.2	3.8	14.80	.....
	{Poor, 4	3,195	1,315	1,880	1:1.4	61.5	25.3	72.7	27.3	13.34	.....
1920	{Good, 6	5,930	1,615	4,315	1:2.7	58.3	23.3	99.3	.7	16.29	.....
	{Poor, 6	4,750	1,380	3,170	1:2.0	60.8	25.0	69.8	30.2	11.88	.....
1921	{Good, 6	8,395	2,103	6,292	1:3.0	.....	26.3	44.8	55.2	10.72	.....
	{Poor, 6	5,302	1,563	3,739	1:2.3	.....	30.8	15.0	85.0	8.59	.....
1922	{Good, 6	3,798	1,117	2,665	1:2.4	54.6	17.6	93.2	6.8	18.02	3.86
	{Poor, 6	2,490	913	1,577	1:1.7	57.8	22.0	61.5	38.5	12.54	2.32
1923	{Good, 8	8,646	2,016	6,630	1:3.3	59.7	23.9	79.9	20.1	11.65	1.93
	{Poor, 7	6,706	1,781	4,925	1:2.8	60.0	26.7	49.2	50.8	10.49	1.54
1924	{Good, 8	6,542	1,284	5,258	1:4.1	58.3	19.2	98.7	1.3	16.57	.....
	{Poor, 7	3,505	1,119	2,386	1:2.1	60.0	20.8	73.8	26.2	12.66	.....

LATER FIELD AND LABORATORY OBSERVATIONS

Field and laboratory observations subsequent to 1919 entirely confirm the earlier ones which induced this study of the cause and prevention of yellow berry.

There has developed a very marked difference in the habit of growth of plants on plots receiving good as compared with poor summer-fallow treatment. Although the rate of seeding is the same for all plots, the early-plowed and well-tilled ones invariably produce a thicker stand of heavily tillered plants of a deeper green color, greater height, and sturdier growth than the plots given late spring plowing and little or no cultivation during the summer-fallow season. Figure 2 was reproduced from a photograph of six plants uprooted April 25, 1921, from an early-plowed well-tilled plot, and of six taken at the same time from a late-plowed poorly tilled-plot. Occasionally soil moisture is insufficient to carry the heavier growth to maturity without damage to yields of straw and grain by burning.

As a rule, however, the good fallow has continued to produce the greater weight of straw and grain; the grain from those plots continues to be less infected with yellow berry, and both grain and straw are substantially richer in protein. These facts are summarized down to 1924 in Table 1.

CAUSE OF YELLOW BERRY

Anyone at all familiar with the history of hard-wheat production will recognize in the preceding statements of facts as observed for Turkey wheat on the tillage plots at Moro, a problem that is as old as the hard-wheat industry. It may be well to refer to comments by European writers on the prevalence of yellow berry in the hard wheats of southeastern Europe, and to those of Australian investigators regarding its appearance in hard Australian wheats. In the United States and Canada, too, more or less notice has been taken of yellow berry in hard-wheat regions, and some speculation has been indulged in as to its cause and remedy. The importance attached to it is directly in proportion, of course, to the cut made by millers under the prevailing market price for normal grain of the same class. For the immediate purpose of this paper it is sufficient to cite only those more recent investigators who have expressed concrete suggestions as to cause and remedy, based upon experimental evidence.

Roberts and Freeman (*5*) in 1908 advanced the suggestion that yellow berry in the hard winter wheats of Kansas "is a 'tendency,' which finds expression in certain strains or races more markedly than in others, and is heritable." In a report of later work by Roberts in 1919 (*6*), it is apparent that he, while still convinced that his earlier suggestion was well-founded, felt obliged to concede that however pronounced heritable tendencies in the direction of yellow berry might be for any one variety of hard winter wheat, soil and climatic factors, operating for or against that infection, were far more pronounced in their effect.

In 1914 LeClerc and Yoder (*4*), of the Bureau of Chemistry, United States Department of Agriculture, mentioned "flinty kernels" in discussing environmental influences on wheat. From the results of then recently concluded experimental work they concluded that climate is the most potent factor in determining both the physical and the chemical properties of wheat. In reporting more recent work by the Bureau of Chemistry on the effects of nitrogen fertilization on wheat, Davidson and LeClerc (*1*) conclude that inorganic nitrogen applied at the time of heading is a very effective agent in preventing the development of yellow berry in hard wheat. Expression is also given by these investigators to the view that possibly the color of the flinty kernels is due to the residual effect of the basic element of the nitrogen compound.

Headden (*2*) comments upon the tendency of some observers to charge up the cause of yellow berry to excessive rainfall and, in irrigated districts, to excessive irrigation. He produced field and laboratory data to prove that variation of soil moisture within rather wide limits under irrigation practice is without effect on the development of yellow berry. He expressed the opinion, also, that far too much importance has been attached to climatic factors as

causal agents of yellow berry, and cites his own experiments in Colorado to prove that the all-important factor in the production of uniformly hard, flinty wheat, uninfected with yellow berry, is a proper ratio of available soil potassium and nitrogen. Headden's work was almost entirely with hard spring wheats.

The senior author of this paper and associates (*3*) at the University of Idaho have shown also that the hardest of hard spring wheats is both possible and reasonably to be expected with irrigation on improved sagebrush lands of the Snake River Plains region. ("Improved lands" in this connection means lands enriched with available nitrogen by the growth of alfalfa and red clover, for which crops the sagebrush lands are remarkably well adapted.)

One might readily conclude from the field and analytical data already given that there is already sufficient evidence that well-tilled plots produce better-nourished plants. The higher yields of grain and straw from these plots, and the higher content of nitrogen in both, simply mean that substantially larger amounts of nitrogen and, presumably, of other elements have been furnished the growing crop on well-tilled plots each year as far back, at least, as 1918. It has appeared worth while, however, to attempt at least to get down to the fundamental reasons for this result.

DRY-FARMED LANDS LOW IN ORGANIC MATTER

It has been remarked that the inherent weakness of the soils in this particular dry-farmed region lies in their low content of organic matter. Unless nitrogen fixation takes place much more extensively than is surmised, crops must depend on this small supply of organic matter for their supply of nitrogen. There is no evidence that this condition is at present the limiting factor in crop production. It is conceivable, however, that long before the soil's native supply of nitrogen is depleted sufficiently to make of that element the limiting factor in crop production, the lack of a liberal supply of that element in readily available form at all times might be reflected in some physiological disturbance in crop growth—as yellow berry in Turkey wheat.

The denser and more vigorous growth of plants on the early plowed and well-tilled plots might be due solely to a greater soil-moisture content conserved by better tillage, or it might be due to a larger content of soil nitrogen and other elements of plant food in readily available form. The first-mentioned condition might be, in part at least, the cause of the second, but only the larger supply of available plant food elements, particularly of nitrogen, could stimulate the greater production of protein in the grain and straw of the plots which make the denser growth. Soil-moisture determinations on these plots have been made systematically almost from the initial layout. In 1920 it was decided to try to find out whether differences already noted in yields, and more particularly in the character of the crop, could be corelated with significant differences in available soil nitrogen and possibly other elements of plant food. The summarized analytical data for five years seem to give a substantial basis for definite conclusions as to the cause of yellow berry in dry-farmed Turkey wheat.

MOISTURE AND NITRATE ACCUMULATIONS IN SUMMER FALLOW

Obviously the mass of analytical data resulting from work so wide in scope was necessarily great. However, each year the data were reduced to graphic form, and the trend of events was closely studied. As the year-by-year results warrant the same general conclusions, they are presented here in summarized graphic form only.

In all cases the composite samples taken from the plots for moisture and nitrate determinations represent four borings as the plots were

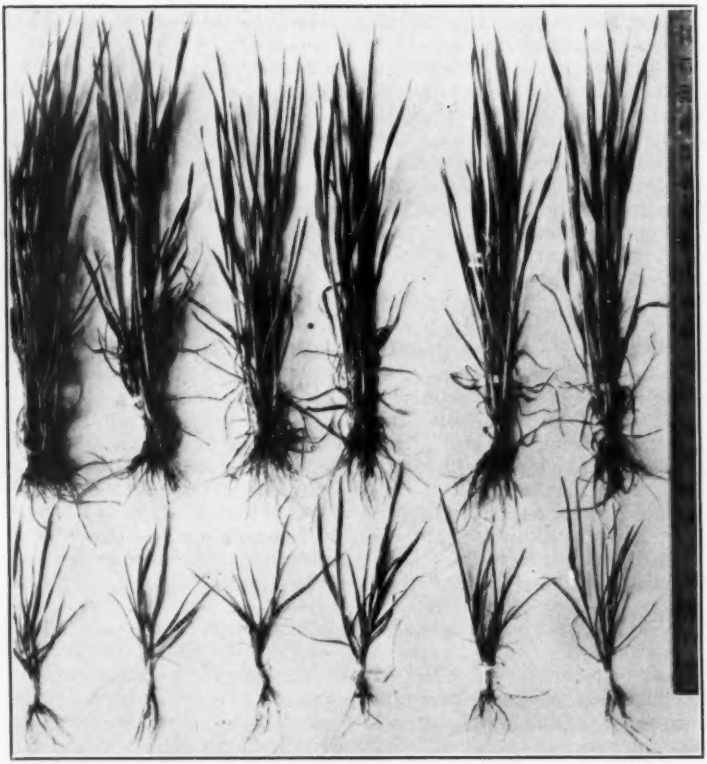

FIG. 2.—Growth of plants on two different plots. The plots were adjacent (see fig. 1), but received different summer-fallow treatment. These plants were uprooted April 25, 1921. *Top row:* Representative plants from plot 383, plowed April 1, 1920, and given frequent cultivation during the fallow season. *Bottom row:* Representative plants from plot 382, plowed June 1, 1920, and given no cultivation during the fallow season

traversed from end to end. Graphs for each of the 6 feet might have been reproduced here, but those representing the averages presented are sufficient for the immediate purposes of this paper.

In Figures 3 and 4, beginning with fallowed and cropped land, respectively, is shown the course of events with respect to soil moisture in 6 feet of typically "good" and "poor" fallowed plots through a continuous period of five years.

One must note the greater decline in soil moisture in the poorly tilled plots during the period of no rainfall during the summer-fallow season; the sharp rise in soil moisture in all plots coincident with the late summer and fall rains; the near equality of soil moisture in all plots in the early spring of the cropped year; and the very sharp decline in soil moisture in all plots accompanying crop growth to near equality at harvest time.

The slightly greater moisture content of the poorly tilled plots during the cropped season from seed formation to harvest time is

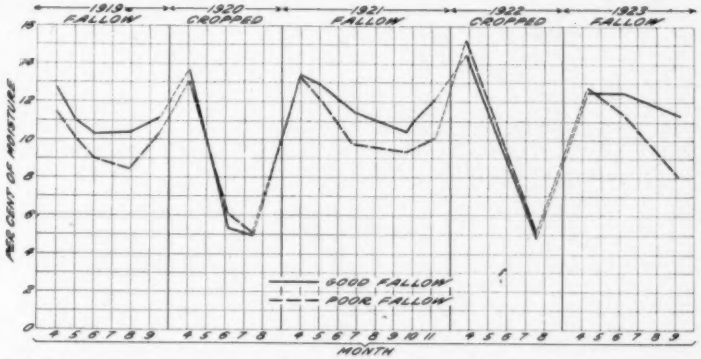

FIG. 3.—Soil moisture in 6 feet of typically "good" and typically "poor" fallowed plots through a continuous period of five years. Fallowed in 1919

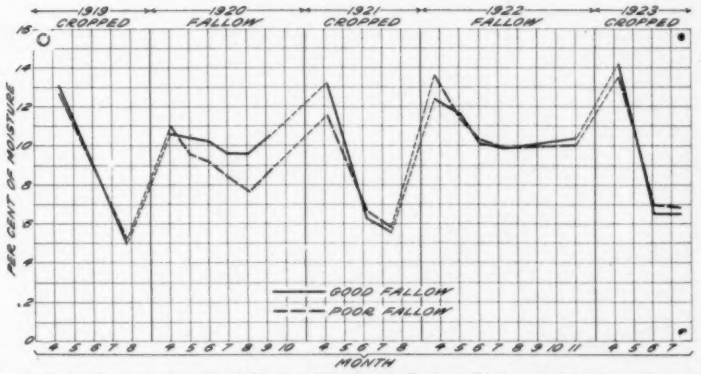

FIG. 4.—Soil moisture in 6 feet of typically "good" and typically "poor" fallowed plots, through a continuous period of five years. Cropped in 1919

characteristic. So also is the greater percentage of yellow berry in the harvested grain of these plots. It is believed, however, that the first difference, however characteristic, is entirely too small to account in any appreciable measure for the latter. There seems to be more evidence for supposing that the greater moisture content of the well-tilled plots during the summer-fallow season is conducive to greater bacterial activity, the sum total of which is a larger amount of available plant food in readiness for the succeeding crop.

NITRATES IN THE CROPPED PLOTS

The first attempts to establish the correctness of this view were made in 1920. Differences in habits of growth previously noted were so very marked in the early springtime that determinations of soil nitrates on the cropped plots seemed promising of results that would establish a connection between available soil nitrogen and vigor of crop growth. From Table 2 it will be seen that although the actual amounts of nitrogen in nitrate form were low on April 15 and May 5, the well-tilled plots had decidedly the larger accumulation of excess available nitrogen. By May 19 the differences were practically negligible as the natural result of the more exhaustive feeding of the denser growth on the well-tilled plots. It is evident that by May 19 there was no appreciable excess of available nitrogen in either well-tilled or poorly tilled plots. All subsequent nitrate determinations were confined to plots actually in summer fallow. This introduces some difficulties also in the matter of interpretation and correlation of analytical data.

TABLE 2.—*Average nitrate content per foot of soil to a depth of 6 feet in plots under crop in 1920*

[Some plots have from the start received good tillage while under summer fallow; others have been given little or no tillage during the fallow season]

Date of sampling	Good tillage		Poor tillage	
	Plot No.	NO_3, parts per million	Plot No.	NO_3, parts per million
Apr. 15	481	3.88	482	1.03
	581	5.16	582	1.66
	681	4.98	682	1.66
	483	3.00	484	2.19
	583	4.04	584	2.02
	683	3.16	684	2.49
Average		4.04		1.84
May 5	481	15.5	482	5.75
	581	7.5	582	3.80
	681	10.0	682	4.60
	483	7.25	484	7.02
	583	11.30	584	3.88
	683	8.40	684	4.57
Average		9.99		4.94
May 19	681	5.67	682	4.65
	683	4.25	684	4.10
Average		4.96		4.38

NITRATE ACCUMULATION IN FALLOWED PLOTS

Figures 5 and 6 were derived from moisture and nitrate data indicative of the average foot for a depth of 6 feet in well-tilled and poorly-tilled plots from 1920 to 1924, inclusive. Figures 7 and 8 were derived from data of the same kind for the first foot of soil. Inasmuch as one will arrive at essentially the same conclusions from a study of both sets of figures, comment will be made on only the latter. From the figures at least four facts can be read:

(1) There is a near equalization of nitrates in all plots in early spring; (2) the period of most rapid nitrate formation roughly coin-

cides with the period of soil moisture decrease and temperature rise; (3) the greatest accumulation of nitrogen in nitrate form at the end of the summer season is always in the well-tilled plots—those of higher moisture content; (4) the accumulation of nitrates at the usual time for fall seeding is the equivalent of very substantial applications of sodium nitrate.

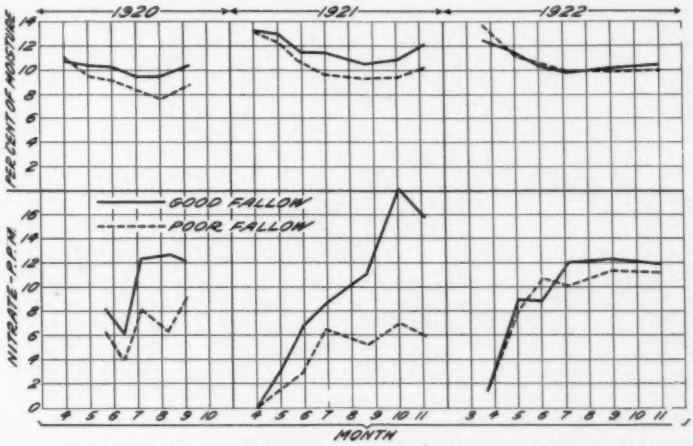

FIG. 5.—Average accumulation of moisture (upper graphs) and of NO_3 (lower graphs), per foot of summer fallow, to a depth of 6 feet, 1920 to 1922, inclusive, in well-tilled and in poorly-tilled plots

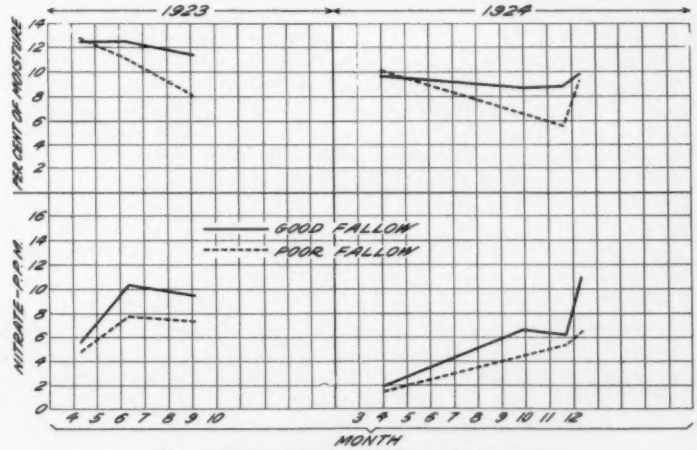

FIG. 6.—Continuation of Figure 5, seasons of 1923 and 1924

It might reasonably be expected that the better tillage treatment increases also the accumulation of other elements of plant food in water-soluble form at seeding time. Only the most delicate methods employed in soil analysis could be made to throw light on that point. The cryoscope seemed to offer the necessary delicacy. In Table 3

are data obtained on the lowering of freezing points by water suspensions of soil from samples taken in three cores to a depth of 24 inches in well-tilled and poorly-tilled plots summer fallowed in 1924. Since (with the exception of samples taken on August 18) the greater

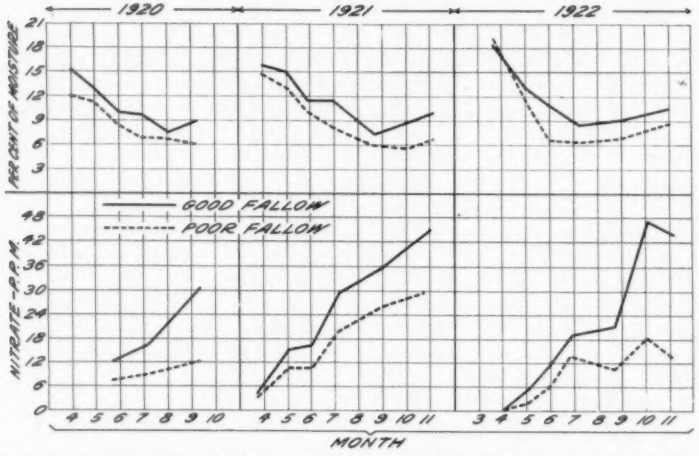

FIG. 7.—Average accumulation of moisture (upper graphs) and of NO_3 (lower graphs), for the first four months of summer fallow, 1920 to 1922, inclusive, in well-tilled and poorly-tilled plots

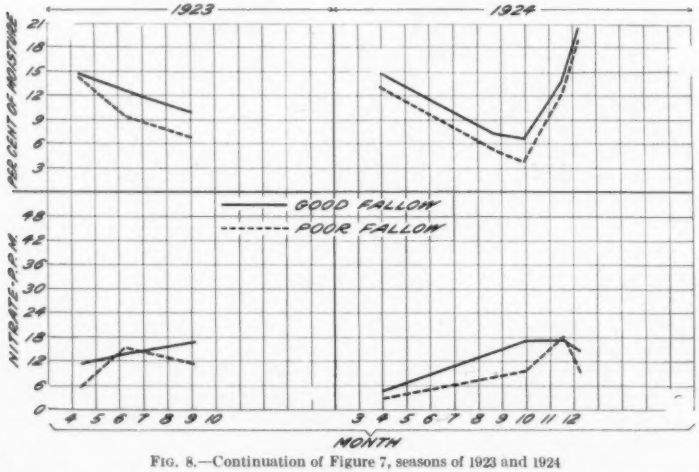

FIG. 8.—Continuation of Figure 7, seasons of 1923 and 1924

depression of the freezing point always occurred with soils from the well-tilled plots, the conclusion that tillage of the summer fallow tends to increase the accumulation of water-soluble plant food appears to be reasonably well founded.

TABLE 3.—*Cryoscopic determinations of water-soluble substance in soils from well-tilled and poorly-tilled fallow plots*

Date of sampling	Depth of core	Depression of freezing point		Date of sampling	Depth of core	Depression of freezing point	
		Well-tilled plots	Poorly-tilled plots			Well-tilled plots	Poorly-tilled plots
1924:	*Inches*	° *C.*	° *C.*	1924—Continued.	*Inches*	° *C.*	° *C.*
July 21	0 to 9	0.009	0.007	Oct. 16	0 to 9	0.007	0.000
	10 to 18	.002	.000		10 to 18	.006	.002
	19 to 24	.006	.002		19 to 24	.005	.001
Aug. 18	0 to 9	.005	.007	Nov. 19	0 to 9	.011	.009
	10 to 18	.007	.008		10 to 18	.001	.001
	19 to 24	.002	.000		19 to 24	.005	.000
Sept. 17	0 to 9	.009	.005	1925:			
	10 to 18	.025	.010	Apr. 6	0 to 9	.012	.008
	19 to 24	.006	.001		10 to 18	.007	.004
					19 to 24	.009	.002

When attempt is made to correlate these facts with the field and laboratory data given in Table 1, difficulties at once arise. Not always do years of greatest yields immediately follow those of greatest nitrate accumulation in the fallowed plots at seeding time. Nor can one with much confidence attribute this fact to differences in moisture retention by the fallowed plots; for, if reference is again made to Figures 3 and 4, it will appear that, notwithstanding the differences in moisture retention during the fallowed season, a remarkable uniformity prevails in the moisture content of all plots at the beginning of the crop season. As a matter of fact, low yields invariably have followed climatic conditions that were unfavorable for germination and emergence of grain in the preceding autumn. The outstanding fact to be noted here is that as between plots of good and poor tillage, grain sown on those of good tillage in the very early stages of growth had a substantial advantage over grain on those of poor tillage in the matter of soil moisture, readily available nitrogen, and perhaps of other readily available plant-food materials as well. As these plots invariably produced the highest yields of grain and straw, which were substantially the richest in protein, the correct inference would seem to be that yellow berry is a manifestation of nutritional disturbance caused by a lack of sufficient nitrogen and perhaps of other essential elements for vigorous plant growth.

IMPORTANCE OF NITROGEN IN DRY-FARMED REGIONS

Simple calculations based upon field and analytical data given in Table 1 put in concrete form two very important facts: (1) Very considerable amounts of nitrogen are being removed annually from all of these plots by the wheat crops they produce; (2) the better the tillage methods practiced on the summer fallow the greater the amounts of nitrogen removed by the succeeding crop.

If the cause of yellow berry in Turkey wheat is correctly surmised from the work reported here, a certain amount of control by summer-fallow tillage appears to be feasible, just so long as soil nitrogen is not too sharply the limiting factor in crop yields. However, one may not reasonably expect control to average well up towards 100 per cent over a long term of years.

The nitrogen problem for dry-farmed lands is perhaps the biggest problem that faces the experiment station at Moro. The removal of nitrogen from the plots at Moro is indicative of what is taking place in the surrounding country, and the question is, how long can the process continue.

SUMMARY

The work reported here is subordinate to a larger project which seeks to determine the influence of variations in summer-fallow tillage on wheat yields.

Thorough tillage of the summer fallow, in contrast to neglect or poor tillage, results in larger yields of grain and straw, both of which are substantially richer in protein and the grain correspondingly less infected with yellow berry.

Since the accumulation of nitrate nitrogen, and seemingly of other water-soluble plant-food substances, at seeding time is always greatest in the well-tilled fallow, it is concluded that yellow berry—low-protein wheat—is the manifestation of nutritional disturbances resulting from insufficiency of nitrogen and other elements of plant food for adequately meeting the requirements of a normally developing crop.

As no means appears feasible as yet for fortifying dry-farmed lands against the gradual depletion of their native supply of nitrogen, it is reasonably certain that high efficiency in the control of yellow berry can not be maintained indefinitely by tillage of the summer fallow, no matter how thorough the tillage may be.

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WESTERN LARCH NURSERY PRACTICE[1]

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INTRODUCTION

Although western larch (*Larix occidentalis*) is at present of minor importance in the northern Rocky Mountains and reproduces itself naturally and abundantly on many burned areas, it has not been entirely neglected in planting studies in this region. On lands in need of artificial reforestation there are likely to be some sites better suited to larch than to any other species. To establish larch on these sites it is necessary to produce seedlings or transplants in a nursery, for effective methods of sowing seed directly in the field have not been developed. With this need in view, studies were made to determine the best season for seed sowing, the proper depth to cover seed in seed beds, and the best method of sowing. Two phases of the work of caring for seed beds were also studied, viz, water and shading.

Most of the work was done at a small nursery at Priest River in northern Idaho. Some tests of time and amount of seed for sowing, as well as part of the shade study, were made at Savenac nursery in western Montana. The natural soil at the Priest River nursery, which occupied a gentle southerly slope, was a rather heavy silt, but was made more friable by the addition of fine, sharp sand. The beds, each of which was 48 square feet in size, were protected only with wire screen frames, except in the shade tests, in which lath shade-frames giving half-shade were used. Examination of beds and seedling counts were made at frequent intervals during the periods of rapid germination and less frequently later in the season.

The seed used at Priest River was collected in 1911 on the Colville National Forest in northeastern Washington, and that used at Savenac nursery was collected in 1917 on the Kootenai National Forest in northwestern Montana. Greenhouse tests showed the vitality of the Colville seed to be 15 per cent and that of the Kootenai seed 19 per cent. Had the seed been of better quality the differences in germination resulting from the various nursery treatments probably would have been greater, showing more clearly the relative values of the treatments.

Because good seed and poor seed can not readily be distinguished and separated, the seed from which samples for sowing were to be taken was mechanically mixed in such a way as to distribute the

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poor seeds evenly in the samples. Then, by considering the percentage of viability of a pound of seed, the weight of a sample that would contain the desired number of good seeds was computed to centigrams.

TIME TO SOW

In the spring of 1913, at the Priest River nursery, three plots were sown that afforded a comparison of the results from early and late sowing. Two plots were sown on May 1 and one on June 6, each plot 4 square feet in area. One-twelfth of a pound of seed, computed to contain 1,200 viable seeds, or 300 good seeds per square foot, was sown broadcast on each plot and covered with a half-inch layer of sand.

Germination was slow in all plots, but the plots sown in May did better than the one sown in June, producing about 70 per cent of their total germination in June and July and the rest in August and September. In the June-sown plot only 5 per cent of the total germination had taken place by August 1. Earlier germination permits a longer time for development during the season, and greater development may fairly be expected to carry with it greater resistance to unfavorable natural influences. Measurements of roots, stems, and needles clearly showed the greater development of the older seedlings, but counts of survivals did not indicate so clearly that these seedlings were correspondingly more hardy. Although the uniformly low germination and survival did not permit contrasts, the results suggested that spring sowing should be as early as possible, and that because the seed is naturally slow in sprouting, fall sowing may be the best plan.

This suggestion is supported by more recent tests at the Savenac nursery. One bed 48 square feet in area was divided into 14 plots of equal size. Ten of these plots were sown on five different dates in 1919, from August 28 to October 8; and 4 on two dates the next spring, April 16 and May 5. Each plot contained 1,000 seeds and was covered with a quarter-inch layer of sand. During 1920 the average total germination in the fall-sown plots was 17 per cent, and 5 per cent for the spring-sown plots. An additional 11 per cent of the spring-sown seeds germinated in 1921, but no more of the fall-sown seeds sprouted. The bulk of the first-season germination in the fall-sown plots occurred in May and was complete in June, whereas the bulk of the first season germination in spring-sown plots occurred in June and July and was not complete until the end of August. Very similar results were obtained again from two more experiments sown in the fall of 1920 and the spring of 1921, and again in the fall of 1921 and the spring of 1922.

All of this more recent work considered time and amount of germination only. With native species generally, prompt and complete spring germination is essential. One way of attaining this result that was not tried in these experiments has since been reported successful in actual practice. Soaking seeds in water for five days swells them with little sprouting. When such seeds are sown in the spring their germination seems to be as prompt and complete as that of fall-sown seed. It is more difficult to broadcast soaked seed uniformly, however, and there is danger of sprouted seeds suffering from exposure during sowing.

Although the total germination in all these experiments was low, fall sowing had definite advantages as compared with spring sowing. Fall sowing not only resulted in the season's germination being more prompt, but also in fewer seeds holding over until the following year. Even counting these tardy and therefore less desirable individuals, spring sowing did not seem to insure larger germination than fall sowing.

DEPTH OF COVER FOR SEED

The depth to which western larch seed is covered is regarded as especially important, for the seeds are compartively small and seem to be poorly supplied with food. From results of previous experiments sand was selected as the best material for cover. Sand reduces losses from damping-off, increases the rapidity and amount of germination, provides for better water absorption, and results in more uniform seedling stands.

In 1913, data were gathered on the effects of depth of cover in connection with a comparative test of broadcast and drill sowing. Six plots were sown for each method of sowing, each plot having four linear feet of drill or one square foot of sowing surface. Sufficient seed was sown to provide 300 good seeds per square foot or 4 feet of drill. Each plot was covered to one of these depths: ¼, ⅜, ½, ⅝, ¾, and 1 inch.

Clean, sifted, undecomposed granite sand was used. Depth of covering was gauged by toothpicks stuck in the sowing surface so that the ends projected to a height equal to the depth of covering desired. Sand was leveled off even with the top ends of the toothpicks, which later served to indicate the extent to which the cover was washed away in watering the beds. Frequent sprinkling kept the surface moist.

The seed was sown June 6. Germination was counted weekly until September, and biweekly until October 14. It started slowly in July, and was earliest and most rapid under the lightest (the ¼-inch) cover, but by the middle of August the ½-inch cover had taken the lead, with ⅜-inch second and ¼-inch third, and this order was maintained for the rest of the season. The difference in total germination for these three depths at the end of the season, however, was so small that it indicated no clear advantage for any of the three. Germination under the three heavier covers, ⅝ to 1 inch, was decidely slower and less in total amount, and the inferiority was so great as to throw these three classes out of consideration.

Although the ¼-inch and ⅜-inch plots were much alike, a progressive decrease in survival occurred in the other plots at the end of the first season with increase in depth of cover; for the heavier covers survival dropped abruptly. The hold-over germination, or those plants sprouting the following season, showed much the same relationship in survival. It was also clearly evident that the method of sowing affected this result in no way. For each method of sowing, germination and survival were uniformly better with the three shallower covers than with the deeper covers.

It was decided that ¼ inch was probably the optimum depth of sand cover, but that in practice it might be well to aim at obtaining ⅜ inch with an allowable range from ¼ inch to ½ inch. Covers shallower than ¼ inch obviously are desirable, if the results are satisfactory, but tests have not been made with such covers and nursery practice has been guided by the above experience.

METHOD OF SOWING

The experiment just described, made primarily to study the effect of different thicknesses of sand covering, also afforded a comparison of the two common methods of sowing seed in forest nurseries, broadcast and in drills.

The peak of the germination period occurred on August 5. At that time the drill-sown seeds showed a more rapid rate of response than the broadcast seeds. One way to gauge this rapidity is to express total germination in terms of percentage of the number of seeds sown by each method and compare the figures. The difference in favor of drilled plots varied from 6.7 per cent to 22.5 per cent and averaged 13.6 per cent for six lots; but by August 12 this average difference was reduced to 3.5, and by the end of another week, as the season neared its end, it had almost disappeared.

Total germination is important as well as rapidity of germination. The drilled plots showed larger totals than the broadcast plots in four out of six cases. Each of the two exceptions was a plot with thin sand cover. One was the plot having the least cover applied, and the other was one in which the sand had been washed to one side in sprinkling. With all but the shallowest cover, the total germination of the seed sown in drills tended to be greater than that of the seed sown broadcast, and this tendency was more marked with increase in the depth of cover. A possible explanation of this is that the combined lifting power of numerous seedlings located in a narrow strip was more effective than that of a similar group of seedlings scattered over a larger area. It may be that the lifting and fracture of the cover along the line of the drill allowed a larger number of the naturally weaker seedlings to emerge. The smaller size of western larch seedlings would make them more responsive to minor soil differences of this nature than are pine seedlings.

In the periodic examinations of the plots, most of the losses were attributed to damping-off during August. As resistance to damping-off increases with age and vigor of the seedlings, there would be reason to expect a greater loss in the broadcast plots where germination was less prompt and seedlings of susceptible ages were exposed to infection for a longer period; but, on the contrary, seedling vigor as a whole seems to have been greater in the broadcast plots. This was because fewer of the naturally weaker individuals had emerged from the soil. At the end of the first and second seasons the survival in deeply covered plots was higher from broadcast sowing, while the survival in lightly covered plots was slightly higher from drill sowing.

On the basis of these experiments it was decided, at least where it was not difficult to obtain water for irrigation, that western larch seed should be sown broadcast rather than in drills. While there

is little difference in germination or survival at the desirable shallower depths of sowing, broadcasting tends to eliminate the weakest plants, and it is at the same time the cheaper method. Broadcasting requires less time for soil preparation and sowing; it utilizes the space more completely, and costs less in weeding, watering, and general care.

WATERING SEED BEDS

To obtain information for the guidance of nurserymen as to amounts and intervals for watering, and for rangers and others who might produce small quantities of planting stock in localities where artificial watering would be impossible or very costly, three tests of drill-sown seed were planned: (1) Surface cultivation without artificial watering, (2) surface cultivation with moderate watering, and (3) heavy and frequent watering without cultivation.

On May 6, 1913, 3 beds, each 24 square feet in area, were sown. Drills were spaced 3 inches apart, and were sown with amounts of seed computed to contain 100 good seeds to each foot. Seeds were covered ¾ inch deep with sand. Germination was counted once a week in 1913, and once in the fall of 1914.

All beds were watered equally during the period of germination and until August, because the purpose was not to affect germination, but to influence later development. The three test lots thus obtained a fairly even start before August 7, when differentiation in treatment began. For convenience these lots and beds will be referred to as A, B, and C.

Lot A was given no artificial watering after August 7. During the first season it was cultivated after each rain and on every fourth day. During the second season it received the same treatment, except that cultivation between rains was reduced to a frequency just sufficient to maintain a dust mulch.

Lot B was given two quarts of water per square foot every fourth day the first season, and the second season it received enough water artificially applied to make a total of 0.75 inch per week when added to the rainfall for that week. It was cultivated after each rain and watering.

Lot C was not cultivated at any time. It was given one quart of water per square foot every other day the first season, and the second season it was sprinkled enough in addition to rainfall to provide a total of 0.75 inch of water during each half week.

Artificial watering was done on Saturdays and alternate Tuesdays and Wednesdays. When rainfall alone exceeded 0.75 inch during the specified periods for lots B and C, no watering was done, the excess being noted but not carried forward to apply on the next period. Every Monday morning during the second season soil moisture samples were taken from each bed at four depths: Surface inch, 2 to 6 inches, 7 to 12 inches, and 13 to 18 inches. The moisture content in the unwatered, cultivated bed (A) was about 15 per cent lower at all depths than in either of the other two, but differences in moisture between the two artificially watered beds (B and C) were small. The minimum amount of soil moisture, 14 per cent, occurred in bed A; the average moisture content of B and C beds was about 55 per cent, and maxima were near 70 per cent.

Part of the stock was transplanted in November of its second season and the remainder the next April. At the time of fall transplanting 100 plants were mechanically selected from each lot, and lengths of roots, stems, and leaves, and diameters of stems at the ground line, were measured. Averages from these measurements are useful in judging the relative development of plants resulting from different methods of culture. It was found that seedling development was greatest in the bed receiving only cultivation (A), that it was intermediate in the moderately watered bed (B), and was poorest in the bed receiving heavy watering (C). The percentage of trees which survived transplanting was highest, 39 per cent, for the moderately watered stock (B); intermediate, 35 per cent, for the cultivated stock (A); and lowest, 8 per cent, for the heavily watered stock (C). The cultivated stock (A) showed the best development after a year in the transplant bed, with moderately watered (B) and heavily watered stock (C) decidedly poorer.

From these results in drill-sown beds, cultivation alone appeared to be better than either moderate watering with cultivation or heavy watering alone for the production of well developed two-year-old larch seedlings. By drill sowing and cultivation it should be possible in ranger nurseries to produce good larch stock without irrigation. For larger nurseries where seeds are sown broadcast, these experiments indicate that moderate watering is best.

SHADING OF SEED BEDS

On June 6, 1913, and again on May 5, 1914, three plots were sown broadcast to test the effects of one-half and one-quarter shade as compared with full sunlight on seed beds. Enough seed was sown to provide an estimated 300 good seeds per square foot. Counts were made weekly during the period of active germination and biweekly thereafter until the end of the season. In the fall of 1914, samples of stock from all sowings were taken up for measurements of length of roots, stems, and leaves, and diameter of stems at the ground line. Seedlings were transplanted in November, 1914, from each of the plots, with one exception. No trees were moved from the half-shaded plot shown in 1913 because of damage from frost heaving. Survival and development of transplants were recorded in the spring and fall of 1915.

Total germination was greatest under half shade and least under no shade with plots sown in June 1913, but this result was exactly reversed with plots sown in May, 1914. In both years the seedlings started to come up first in unshaded plots, but in the case of the later (June) sowings the shaded plots caught up. This seems to indicate that the seeds are benefited by full sunlight when they begin to sprout, especially early in the season when insolation is less intense, but that when germination is delayed the majority of seedlings will come up only under shade. Of course, survival and development must also be considered.

Because of the inherent slowness of larch seed when spring sown, it would be natural to expect that shade during May and June, even if it made germination still slower, would benefit survival of the

youngest seedlings through its action in reducing transpiration and in keeping the surface soil cooler and more uniformly moist. Shade during these two months, however, appeared to be more detrimental than beneficial because of greater losses from damping-off under the shade frames. Shade on these beds after the 1st of July seemed to benefit survival regardless of whether the seeds sprouted early or late, and half shade was more beneficial than quarter shade. In development during the first year, the trees receiving quarter shade were very slightly larger than the others; after the next year the best developed trees were found in beds having no shade during the second season.

These results were supported by the outcome of some similar work at Savenac nursery. Two 4 by 12 foot beds were sown there on May 18, 1914. One was given no shade at any time, and the other was given half shade from July 1 until the close of the first season. Although the unshaded bed contained more seedlings than its companion on June 22 before either bed was shaded, it fell behind early in July. Periodic seedling counts showed that shade was having a favorable effect upon midseason germination and survival, which by September 8 amounted to a lead of nearly 10 per cent in number of seedlings.

From the results of tests at Priest River and at Savenac nursery, it is recommended that western larch seed beds sown in the spring be given one-half shade during July and August, but no earlier in the first season and none at all during the second.

REMAINING PROBLEMS

In this series of experiments several phases of larch production have not been considered. The proper density in seed beds, the best time for transplanting, and the most suitable age classes deserve attention. With larch, also, some other factor than variation of density in the seed bed is active in producing extremely irregular stands, and this factor is yet to be determined. During their second season, larch seedlings vary roughly from 1 to 12 inches in height. These extremes are greater than in any other species grown in the nursery; a great deal of culling must be done, resulting in a heavy loss in culled plants.

CONCLUSIONS

Larch seeds should be sown in the fall, to avoid hold-over germination, but principally because the first-season germination comes more promptly from seed sown the previous fall.

Present practice aims at ⅜ inch of sand covering, with allowable variations from ¼ to ½ inch. One-quarter inch or less is probably the optimum thickness.

The preferred method of sowing seed is broadcasting rather than sowing in drills. Broadcasting tends to eliminate the weakest plants, with no net loss in survival under the more desirable light sand covering; also the cost is lower in time taken from soil preparation and sowing, in utilization of space, and in the labor demanded for weeding, watering, and general care.

However, where water for irrigation is difficult to obtain, drill sowing may be necessary. It has been shown that well-developed 2-year-old larch seedings can be produced in the northern Rocky Mountain region in cultivated drills without artificial watering. With broadcast sowing moderate watering is best.

The amount and degree of shade needed by spring-sown seed beds is half shade during July and August, but none earlier than that and no shade at all during the second season.

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